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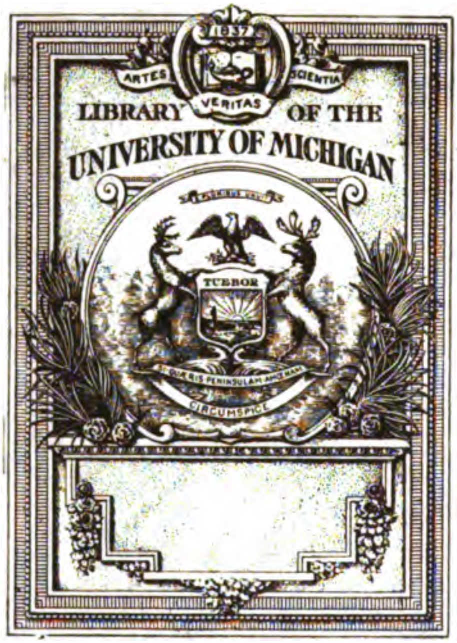
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OUTLINE OF AN ELEMENTARY COURSE IN MICROBIOLOGY

By

JEAN BROADHURST

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OUTLINE OF AN ELEMENTARY COURSE IN MICROBIOLOGY

INTRODUCTION

This is an outline of an elementary course in microbiology, planned not for intensive study, but to cover as wide a range as possible, and so form a general introduction to the whole field of microbiology. Being a two-point course, the class time allotted to it is but 4 hours a week for one half year; the required reading should average at least one hour a week.

Prerequisites. The only prerequisite is an elementary, laboratory course in biology; this should provide a real working knowledge of the microscope. Without further notification each student is personally responsible for remedying any lack in this respect during the *first week* of this course.

Printed directions, with microscopes for practice, may be obtained in the department during the first week. A working knowledge means (1) skill in changing from high to low power (or *vice versa*), using different objectives, oculars, or both; and (2) the power to use intelligently (a) the coarse and fine adjustments, (b) the mirrors, and (c) the diaphragm. It therefore presupposes a sense of discrimination that assures the worker that the adjustment of lenses, mirror, and diaphragm is yielding the best illumination and the sharpest definition possible. Students who have not yet acquired the habit of focussing up (never down) while looking through the tube, or who do not know the approximate focal distance of the different objectives, will need much practice with the microscope. It is also essential that the fingers should be "sensitive" to the fine-adjustment screw, (1) telling instantly when it ceases to affect the focus, and (2) *limiting all movement* of this screw to a *fraction* of a turn. With the oil-immersion lenses it is absolutely essential that one hand should be kept in constant touch with the fine-adjustment screw.

Introductory work with green plants. Since the majority of the students taking this work offer only human physiology as the preliminary biological science, this course begins with a study of the green plant. This is necessary, not only because of the differences in plant and animal cell structure (cell wall, vacuoles, etc.), but because a definite understanding of the physiological activities of green plants is essential to an intelligent view of *dependent* plants, the molds, yeasts, and bacteria, which, naturally, form the major part of this course.

Reasons for wide range of subject matter. Although such a general course forms the best introduction to advanced courses, or for more technical work in microbiology, there are still stronger reasons for a broad rather than an intensive course of study.

First: We live in a world of micro-organisms; "ten thousand on my right hand" is really stating it mildly. Both the favorable and unfavorable activities of these organisms affect us intimately; not only directly, as in disease, but indirectly, as in the case of the soil organisms.

And the control and interpretation of microbial activities is a matter of great moment to every one of us. These are questions, not merely of knowledge, but of application; and the wider the scope of the course, and the more numerous the angles from which the student can view a given situation, the more varied the sources which will help in the solution of any new problems.

Second: Most of the students registering in this course are students in the School of Practical Arts. Their interests are really along three lines: (1) the teaching of the domestic sciences; (2) the practical household and commercial applications of bacteriology; and (3) the relation of bacteria to health and disease. There is really, therefore, no one phase of bacteriology wholly or definitely outside their possible needs or interests.

Demonstrations. We have found that the work could be more economically handled by frankly arranging part of the usual laboratory work in the form of a

demonstration. "Repeating the history of the race" is a fetish that the biological sciences are slow to outgrow. We no longer shape our courses in mathematics, the languages, etc., solely on this principle. We are sane enough there to accept many of the generalizations, principles, and procedures already part of "the history of the race." In fact, "the history of the race" is an inheritance, and should be utilized as such, and not as a fortune which each must accumulate all over again for himself by ha'penny additions. There are numerous ridiculous and pathetic instances of the waste of time, energy, and interest, due to blind adherence to this catchy phrase. It is, moreover, insulting to the intelligence of a college student to imply, for example, that she must actually boil two thermometers to realize that 100° C. is the equivalent of 212° F. It is equally so to demand that she must individually inoculate various strains of bacteria into *all* the different media to appreciate that bacteria vary in their power to use the many foods or media, and that these media may have differential value.

Careful labelling, with interspersed questions, comparisons, etc., makes it possible to bring before the students a large amount of illustrative material with a minimum of student effort, and yet retain most of the benefits commonly accredited to laboratory work itself (relative values, selection, etc.).

It has, therefore, for several years, been our custom to reserve for each period one or more tables for illustrative material related to the topic of that day. For example, in the laboratory period the students individually stain and examine one cheese microscopically; but the demonstration includes slides of other well-known cheeses, and a set of books and pamphlets dealing with cheese-making, etc. The student learns how to make smears of such substances ("fixing" in alcohol, removing the fat with ether, etc.), but there is absolutely no use in having her make and stain smears of each cheese, particularly as the organisms seen at this end stage are not necessarily those *primarily* concerned in cheese-making. And repetitions of that kind (one well-known college demands the microscopic examination of sixteen cheeses!) are valuable only in gaining technique; that, however, is one of the least of the values of an *elementary* course in microbiology. The student would gain much more by trying for one or two cheeses several methods of fixing organisms to a slide, and realizing what it is that "makes them stick, anyway," by staining with and without a fat solvent, or by trying differential stains on such smears. The first method can mean but little more than a statistical enumeration of the dead forms seen; the latter means a vital conception of micro-organisms, including the morphological but emphasizing the physiological and chemical properties and characters.

Running the demonstration *with* the laboratory period has for large classes three disadvantages: (1) With a limited number of oil-immersion lenses, needed at the same time for the demonstration and the regular laboratory work, long waits must occur. (2) This combination hurries unduly the slower students and often, also, the more careful and more thoughtful workers. (3) The preparation and labelling of a large illustrative series, including the adjustment of microscopes, takes a great deal of time. If several sections of one class are necessary, this usually means repetition on several different days; it is therefore not only wasteful of the instructor's time, but almost an impossible task.

At the present time we, therefore, reserve part of a day each week for this demonstration work. Its preparation (demanding from two to five hours per week) is still a real task. But it needs to be set up but once a week, 'though water mounts, etc., will need quite frequent attention during that day.

It has also been found helpful to have the demonstrations overlap, repeating each week the last two or three tables of the previous demonstration. The students do not feel so hurried, and absences are less serious. The labels, pamphlets, etc., used during any demonstration are kept in sequence on a side shelf for two weeks following the demonstration, and may be consulted by students who have been absent.

Proportion of lecture and laboratory work. With a single section it would be more desirable to meet twice a week for two hours each time, adjusting each week the proportionate amount of time demanded by the lecture, the laboratory work, and the demonstration. Running several sections means repeating the same lecture five times in three, or even two days — a deadening performance. Now, therefore, but one lecture hour is provided, and that is a general lecture hour for

all the sections. Each section has its own laboratory period of two consecutive hours once a week, thus making, with the demonstration hour, a total of 4 class hours per week. During the last month part of the laboratory period is sometimes used for lecture work.

Loose-leaf outline and note-books. This outline has been printed in loose-leaf form for convenience in classifying or rearranging the material. It is not necessary or even advisable, in any course, to have *all* the related material presented at the same time; that deprives the student of her rightful opportunity. For while most of the related material should be presented together, giving a progressive unity to the course, the work should not be so perfectly and completely classified that its logical arrangement, proportionate emphasis, etc., leaves nothing for the student to do. In this course single exhibits are sometimes placed with later topics, with the query, "Under what main topic does this belong?" Each week should bring new data or a new principle that makes it necessary to revise an earlier conclusion, or which leads to a broader interpretation of former observations. To illustrate: Some weeks after the work on "Vital phenomena," under which topic the students have studied the various effects produced by bacteria in different media, we reach the topic, "water," and such varying results are then seen to be not only different results, but *differential,* making possible the "presumptive tests" of water.

The loose-leaf outline is also more flexible, allowing variation in the emphasis and the content of the course from year to year, or in the various sections (domestic science, nursing). The main advantage of the loose-leaf form is that it facilitates rearrangement, so that the real relationships of the material accumulated during the term may be shown — not merely the chronological sequence of such material. Such rearrangement may be impossible when both sides of the paper are used.

Students are expected to keep note-books of the assigned readings, laboratory work, and demonstrations. The early demonstrations are prepared partly with a view of presenting briefly material that may be needed by a few students, e.g., the metric system, the types of plant cells (parts, shapes, contents). Having thus assured the same general basis for the work, the demonstration notes should be quite individual; not a mechanical copying of the labels, but a selective process; one student picking out for her book the *new* things; another, the material she can use in her high school class next year; while a third may pay special attention to the pamphlets and books of reference which she will need to supplement her library when she returns to her former position.

Reading Notes. Students are not required to buy any text-book. A few copies of each of the reference books are available in the library and in this department. With large classes, it is not possible to have enough of the latest editions (or of the same editions) of the various texts. Page assignments are not given here, therefore, for most of the texts. This is, from the teaching standpoint, desirable; as the ability to select from a given text the important and related material is not the least of educational aims. (See reference lists following this chapter and at the end of the outline.)

The reading should be contemporary with the lectures, demonstration, and laboratory work. It will help greatly to glance each subject *before* it is taken up in class, and then read a second reference book *carefully* after the class work is completed. The reading notes may be mainly covered by marked text-books, if the student chances to own one or more of those required. This means, however, something more than merely underlining selected passages in a book. Marginal notes, insertions (using thin paper for additions, illustrations, etc.) make even a poor text-book quite valuable for later reference — including reviews and examinations. Look through the annotated text-book shown in the first demonstration and note that it is quite possible to (1) bring an old edition up to date; (2) incorporate statements from other authors; and (3) add helpful diagrams, outlines, and experimental results. Note-books, as we all know, are too cumbersome to keep for reference once the course is completed; but an annotated text-book has a much longer life, and may be a real help for several years. During the first few weeks, reading-notes on one assigned reference are examined (see date on bulletin board), and preferred methods illustrated. Word and phrase abbreviations and the value of indenting and of the bracket or brace as quick ways of showing relationships, are included. Long-sentence notes should be avoided; the eye cannot grasp them

readily enough in later reference, and they do not help a student gain speed in reading, or increase her power of selective ability while reading. It is equally important, also, that the notes should not be so brief that they form a bare outline, conveying no information. Compare in this respect the following notes on chlorophyll from the note-books of two different students, A and B.

A. Chlorophyll — cells
— shape
— iron
— function
— use to man

B. Chlorophyll — in plastids in plant cells — varying shape
— without iron — yellow only
— forms sugar, using CO$_2$ and H$_2$O
— starch stored
— uses to man — source all organic food (even proteins indirectly)
— regulates O supply

The form A is valuable — very valuable — if *filled out after the reading is completed;* used in that way its disciplinary value is unquestioned. But notes left in this form are of very little use for later reference or for review; the form B is decidedly better for such purposes.

Having illustrated and criticized the various methods of note-taking once early in the course, no further reference to such mechanical phases of a science course should be necessary in dealing with mature students (juniors and seniors). At the end of the term the note-books are examined with reference to the methods finally adopted by each student; the selection and arrangement* of such notes are two of the most important methods of measuring student ability.

Laboratory Directions. Laboratory directions may be distinguished by the wider spacing between the lines; they are not given in full, except where they differ materially from those given in the standard references cited (simplifications, such as using cheaper substitutions, commercial, clear-meat bouillon, for large classes, because of the war price of the standard peptones).

Laboratory Books. Laboratory books are examined at the end of the term. In them are expected: (1) reading-notes (a fully marked text-book (as described) may be substituted), (2) *selections* from the demonstrations which you think will be of use to *you,* and (3) *all* the laboratory exercises, reports, experiments, etc.

For some reason, writing up an experiment is apparently a most difficult task, even after two or more years of experimental work in high school, chemistry, physics, biology, etc. (1) Necessary details are omitted; (2) the conclusion is not based solely on the results obtained; or, most common of all faults, (3) material belonging in the procedure or body of the experiment is omitted there and omitted or but barely implied in the statement of the result or in the conclusions reached. For instance, lime water may be used to indicate the presence of CO$_2$. The knowledge that it makes a white precipitate with CO$_2$ determines the apparatus to be used, the method of collecting CO$_2$, etc.; all of which belong in the procedure. Yet 85-95% of over 600 students writing up such an experiment did not mention the fact that CO$_2$ and lime water yield such a precipitate until the result or the conclusion was reached, and then often only incidentally. It is not uncommon to have students describe the result as a milky precipitate, and state in the conclusion, "This shows that a green leaf gives out CO$_2$," though the student has nowhere stated that CO$_2$ forms such a precipitate.

During the first month three or four experiments are called for, carefully criticized, and returned. After that (as in the reference readings just described), the responsibility for the form of all later experimental work rests with the individual student, and the laboratory work is not examined again until the end of the term. The results obtained in each lesson are compared in class, and the

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*This does not mean undue emphasis upon the appearance of the page. Rapidity in reading (and note-taking) is itself of value, and appearance should not be so emphasized that it interferes with this valuable acquisition. Nor is there time for recopying notes. This, however, does not countenance extreme carelessness; even in rapid note-taking, consistent methods of indenting, etc., will care for the placing of the material on the page and insure the arrangement of matter so that (1) important topics catch the eye readily, and (2) relationships are evident.

mechanical writing up of these results is within the power of every serious minded student capable of college work. This method of shifting the responsibility upon the student is necessary in dealing with large numbers: it is, also, the only method for mature students; for no *college* course should be merely a series of detailed exercises.

More frequent examination of the note-books would be advisable or necessary in courses where more drawings are required, and where more complicated laboratory material leaves much to the interpretation of the student, such as the study of the tissues in physiology, botany, etc. At the beginning, some drawings of the green leaf, plant cell types, starch grains, etc., are asked for, to insure the power of using the microscope. But aside from the molds, there are very few cases in this course where drawings mean anything. There is, for example, nothing to be gained by having students draw the various types or species of bacteria seen; for it is rare, indeed, to find student drawings that look at all like bacteria.

Lectures. The lectures are given here in brief outline only. With a new and rapidly growing subject, such as bacteriology, such outlines are preferable, unless a new edition can be brought out each year. Moreover, the subject matter given has been already exhaustively and authoritatively dealt with in various text-books, and there is no excuse for adding a poor text-book to the list of good ones now on the market. It is much more important that our students should have a syllabus of this beginning course for their guidance, than that the material included in the course should be placed within the covers of a single book. That would be more advantageous for cramming; but with the more mature students of such varied preparation and experience as ours, it is far from desirable that they should be limited to the same text-book, or that any student should confine her reading to any one text-book. Besides, a text-book by any lecturer is too much like his lectures; students gain much more in having the matter presented in another way in their readings: First, the important facts are to be found in both, and this review of salient facts is itself valuable; second, the variations are likely to be mainly those of method, items of personal experience, or differences in application and in point of view, all of which add greatly to student interest.

Notes on the lectures of this course are not desired. To avoid the too often absolutely continuous note-taking characteristic of college classes, outlines of all these lectures have been provided. Occasionally a date, a proportion, or a similar detail, might be jotted down for future use. If lectures are to be written up fully, students should fill out such outlines outside of class, and get real training from their note-taking. A still better plan, of course, would be to have students make their own outlines (and outlines only) during the lecture, and fill them out afterward.

Historical Review. The chronological arrangement of the important facts in the history of bacteriology is placed at the *end* of the course, instead of the beginning, where the bird's eye view is usually given the student. In a subject dealing with such technical terms or unusual materials, a chronological sequence would be an unintelligible and uninteresting array, and such epoch-making introductions as bacterial stains and solid media would pass unnoticed at the beginning of the term. But if discussed at the end of the course, each discovery or event is seen as preparing the way for another, and the later ones actually click into their places in the whole related scheme.

No better review of the whole course can be imagined than that possible through the discussion of the statements listed in the chronological table at the end of this pamphlet. The logical development of the science is easily shown. Interest also attaches to the three broad periods into which the work falls: (1) the speculative theoretical beginnings; (2) the definite, accumulative second phase accompanied by improvements in the microscope, the introduction of stains and of solid media, and marked by rapid additions to the number of known organisms; and (3) the study of the effects of these micro-organisms upon the tissues of animals and plants, and the reactions against them. We thus progress from (1) theories to the (2) knowledge of the organisms and their activities, and finally (3) to the interrelations between these minute organisms and the rest of the universe, man included.

BOOKS RECOMMENDED FOR BIOLOGY 57

INTRODUCTORY BOOKS

Green Plants

Curtis: Nature and Development of Plants (Part I) (Holt)
Bigelow: Applied Biology (Macmillan)

Yeasts and Molds

Cohn: Bacteria, Yeasts, and Molds (American Book Co.)

GENERAL REFERENCE BOOKS

Look through the following (7) books carefully, noting the author's aim (introduction) and the general style and scope of the book.

Hereafter reference to these books will be made by using the authors' names only. Plan to use each one at least once early in your reference reading, that you may find which author is best suited to your needs.

Park and Williams:	Pathogenic Bacteria and Protozoa (Lea & Febiger)
Jordan:	General Bacteriology (Saunders)
Buchanan:	Household Bacteriology (Macmillan)
Moore:	Microbiology (Carpenter & Co., Ithaca, N. Y.)
Hiss and Zinsser:	Text book of Bacteriology (Appleton)
Marshall:	Microbiology (Blakiston)
Stitt:	Practical Bacteriology, Bloodwork, and Parasitology, (Blakiston)

Which book would you use as a reference for molds? for yeasts? for human diseases? for economic phases of bacteriology? for technique (stains, preparation of media, etc.)? for water examination? for milk examination? as a high school reference book? as a nurse's reference book? for protozoa and other animal parasites? Answer these now; but keep your list for amendment during this course.

THE MICROSCOPE (prerequisite requirements)

Students who are not thoroughly at home with the microscope (see note on p. 1) are expected to become so before this first week ends. If you cannot *readily* change from high to low powers, with *distinct* illumination, read the following beginner's directions, and arrange to come in to practice with a microscope this week. No practice will be given in class hours, as "laboratory experience" is a prerequisite of this course.

The microscopes required for ordinary work are "simple" and "compound."

SIMPLE MICROSCOPE

The simple microscope may be either a pocket lens or one of the more elaborate dissecting microscopes. Microscopes of this class range in magnification from two to forty diameters. Usually, however, for magnification higher than about twenty diameters, the compound microscope is used.

COMPOUND MICROSCOPE

The accompanying labelled picture will help in identifying the more important mechanical parts of a compound microscope: The heavy foot, or *base*, often made in the shape of a horseshoe; the upright *pillar*, which is supplied with a joint, or hinge, allowing inclination of the instrument. This hinge is rarely used. The *stage* is the horizontal support, or shelf-like part upon which the object to be examined is placed, having first been mounted upon a glass slide. The stage is often provided with a pair of *clips*. These are used to hold the glass slip, or object slide, in position. The *mirror* reflects the light through the hole in the stage, and thus illuminates the object slide. The mirror is so arranged that it can be turned in various directions. Immediately beneath the stage, in some instruments, is a revolving plate with holes of various sizes. This is the *diaphragm*, and it is used to regulate the amount of light. In other instruments plugs with holes of different sizes are inserted into the hole in the stage, and serve the same purpose. A third style of diaphragm is the iris diaphragm; examine several

microscopes until you find one made of sliding, overlapping, metal strips. The *arm* of the microscope reaching out from the pillar, supports more or less directly, the *tube*, which in turn carries, partly within itself, the *draw-tube*. The tube may be moved up and down by the rack and pinion — "*coarse adjustment*." More delicate motion is secured by the use of the "*fine adjustment*," which is regulated by a small milled wheel, generally placed at the top of the pillar. Careful work with high powers makes it necessary to readjust the focus constantly; experienced workers keep the (left) thumb and forefinger on the fine-adjustment screw.

The parts of the microscope thus far mentioned are generally included in the term "*microscope stand*." The optical parts consist of *eye-pieces* or *oculars*, which fit into the upper part of the draw-tube, and of much more expensive *objectives*, which screw into the lower end of the tube. A very convenient, though not essential, apparatus is the *nose-piece*, by means of which two or more objectives, revolving around a common center, may be attached to the lower end of the tube, permitting an instantaneous change of magnifying powers.

In the microscopes in use in this laboratory, objectives marked 16 mm., 2/3, and 3, are low-power objectives; those marked 4 mm., 1/6, and 7, are high-power objectives. The low-power oculars are marked 2 (Bausch & Lomb), 1 (Leitz), and ×4, (Spencer); the high-power oculars are marked 1 (Bausch & Lomb), 3 (Leitz), and ×8 (Spencer).

A statement of magnifications possible with the various objectives and oculars is furnished by the manufacturers. Roughly, they range as follows:

Low-power objective and low-power ocular...	50 to 60
Low-power objective and high-power ocular...	90 to 100
High-power objective and low-power ocular...	250 to 370
High-power objective and high-power ocular...	450 to 525

Use of the Compound Microscope

1. Position on the table. Place the stand so that the two arms of the base are directed away from you. Do not twist it to the left or right, but place the microscope at right angles to the body, so that the screws, etc., will always have the same relative position to you, and may be controlled without removing the eye from the ocular position.

2. Nose-piece. See that the low-power objective (2/3, 16 mm., or 3) is directly over the hole in the stage. As it moves into position, you can hear or feel it clink into place.

3. Mirror. Adjust so that light is reflected up through the tube and ocular. Never use direct sunlight. The concave side of the mirror gives brighter illumination (except with the oil-immersion lens, which will be described later).

4. Mounting objects. Place a few fibers of cotton in the center of a *clean* glass slide. With a pipette put on a drop of water. Cover with a *clean cover-glass*.

5. With coarse adjustment, raise the tube until the lower end of the low-power objective is about ½ inch above the stage. Place the mounted slide so that the object is in the *optical axis*, which is for practical purposes the axis of the tube. With the coarse adjustment, lower objective to within ¼ inch of cover-glass. While looking through the tube, slowly raise it until the object comes into view. Such adjustment of the optical parts is called "focussing." Repeat the above directions until you are able quickly to find the focus with the low power. Bubbles of air are often caught below the cover-glass; they may appear as iridescent globes, as black rings, or irregular black-bordered spaces.

6. Never focus downward while looking through the microscope. Instead look directly at the end of the objective, holding the eye level with the stage, when it is necessary to lower the tube.

7. High-power objective. When an objective is in focus with the low power, it is possible, by turning the nose-piece, to bring the high-power objective into the optical axis, and so nearly in focus that only a slight turn of the micrometer screw (fine adjustment) is necessary. Lenses of different makes, however, are not always interchangeable; the length of the objectives may vary so much that the high power may strike the slide or the wet cover-glass as it swings into place. It is always safer, therefore, to raise the tube slightly before swinging the high-power

objective into position. Great care must be taken in order to avoid touching the cover-glass with this objective. Practice changing from high to low power and *vice versa.* Always make sure of your illumination with the low power before trying to use the high power.

8. After using the high-power objective, always turn or lift the nose-piece before removing the object slide from stage.

9. Keep both eyes open and use the right and left eye alternately. If it is very difficult to keep both eyes open, cut a circular hole in a 3-4 inch strip of paper, slip it over the ocular and out under the unoccupied eye.

10. Clean lenses with the Japanese lens paper only, never with filter paper or a handkerchief. Use this paper for no other purpose, and keep it protected from dust; discard all used pieces. If the high-power objective needs cleaning, consult the instructor.

11. Keep the mirror and ocular clean. With a soft cloth wipe dust from the metal parts of the instrument.

12. In removing or carrying the microscope, grasp the pillar below the stage. Hold it in a perpendicular position, or the oculars may drop out.

13. Keep these directions at hand, and review them until you are familiar with the microscope and its workings.

ADDITIONAL MICROSCOPE EXERCISES

Necessity for mounting in water; for using a cover-glass. (Drawings are not required.)

1. Place a few grains of potato starch on a slide. Cover with a cover-glass; examine with both low and high power.

2. Run water under the cover-glass by placing a drop from a pipette at one side. What difference in outline? In definiteness of the surface markings?

3. Remove the cover-glass and study the grains in the water. How does the presence of the cover-glass affect your microscopic picture?

The Oil-immersion lens (Defer until p. 26, no. 3).

1. Note the small lens through which light enters in this objective. Its small size makes it necessary to focus the entering rays so that they do not conflict with each other, and to insure that as many as possible enter the lens. This is done by a lens under the stage, the condenser. Swing it in and out of position, and note the effect upon the appearance of the bacteria on the prepared slide provided.

2. With a lens so convex (or its equivalent in superimposed lenses), the magnification is much increased; but the refraction (see wall chart), which gives increased magnification, means a corresponding loss in light. Few of the light rays pass through the ocular to the eye; this makes the image less distinct than in lower magnifications. It is therefore necessary to see that less of the light rays are lost in their passage from the glass slide to the oil-immersion objective. Ordinarily the rays pass from the slide and cover-glass into the air, then into the lens. Every change into a medium of different density (air, water, glass) means a change in the direction of the light rays, and a corresponding loss — by rays that never enter the objective, or fail to focus harmoniously. To lessen these losses, a drop of oil, having the same refractive index as glass, is placed between the cover-glass and the lens, making a continuous glass-oil medium. The water between the slide and the cover-glass may similarly be replaced by balsam as in permanent mounts. In rapid routine work where smears are made on the *slide,* the cover-glass is often omitted entirely; the drop of oil is placed directly on the smear and the lens lowered into

it. (Good permanent slides can not be made from oiled smears.) Lower the objective, as shown, into the drop of oil, until it spreads around the base. Then focus *up* until the proper focal distance is obtained. (Do not use more oil than is necessary to make the connection between the slide and the lens. The lens in the objective is set in balsam; solvents necssary to remove such oil from the lens may also loosen the lens from its setting.)

I. GREEN (INDEPENDENT) PLANTS (Emphasis on the leaf)

Illustrative material:
- Colored charts:
  - (1) cross section of leaf
  - (2) epidermis with stomates
  - (3) stem and root sections, showing clearly veins, supporting cells, "rays"
  - (4) cells: shapes, parts, and contents
- Skeleton leaf
- Green leaves picked at 7 A. M. and at 4 P. M. and decolorized in alcohol; to be tested with iodine for starch during the lecture

A. Morphology and physiology of stem and root
- (A) Root
  - a. holdfasts
  - b. absorption: water, minerals in solution
  - c. storage: water, food
- (B) Stem
  - a. support: proper exposure of leaves, flowers, and fruit
  - b. storage: water, food
  - c. transfer: importance of route between root and leaf
    - (a) veins
      - 1. upward: water, minerals in solution
      - 2. downward: elaborated foods made in leaf
    - (b) "rays" — across the stem at all levels: water, minerals, elaborated foods
- (C) Relation between cell structure and function, e.g., root hairs: thin wall, increased surface, water reservoir

B. Morphology and physiology of the leaf
- (A) Gross structure
  - a. blade — flat expanse of tissue, increasing
    - (a) exposure of chlorophyll tissue to light, and
    - (b) epidermal area concerned in adjustment of O, CO_2, and H_2O
  - b. petiole — path of transfer, as described for stem
  - c. stipules — often lacking, especially at maturity; may function as blade does (e.g., pansy)
- (B) Microscopic structure and functions of specialized leaf tissues
  - a. epidermis
    - (a) usually one layer of non-green cells
      - 1. holds inner tissues together
      - 2. allows penetration of light
      - 3. protects inner tissues from drying out
    - (b) stomates
      - 1. number
      - 2. structure
        - (1) formed by two guard cells containing chlorophyll
        - (2) effect of turgidity of guard cells on size of opening

- 3. function of stomates
  - (1) passage in and out of O and of CO$_2$
  - (2) H$_2$O eliminated (*not* absorbed) by the leaf
    - —excess water from root
    - —relate to low mineral content of soil waters
    - —water of metabolism
  - (3) advantage of usual position of stomates with regard to sunlight, dust
- (c) epidermal modifications affecting function (hairy, waxy, non-wetable surfaces)

- b. palisade tissue
  - (a) below upper epidermis
  - (b) one or more layers of closely-packed chlorophyll bearing cells
  - (c) function
    - 1. utilization of CO$_2$ (from air) and H$_2$O (from soil) forming a simple sugar
    - 2. requirements for this synthesis of CO$_2$ and H$_2$O
      - (1) sunlight
        - —plants adapted to varying intensities.
        - —sun as source of energy for utilization of CO$_2$
        - —explanatory theories of photosynthesis
      - (2) chlorophyll
        - —held in chloroplasts
        - —soluble in alcohol
        - —iron necessary: color of chlorophyll; synthesis of sugar
    - 3. effect of plant photosynthesis on air content
      - (1) heavy elimination of O (12 atoms for every molecule of sugar formed), and its effect upon the O contents of the air
      - (2) withdrawal of CO$_2$ considerable
        - —important because of O liberated, *not* because CO$_2$ acts as poison
        - —1 yd. leaf surface can use CO$_2$ from 2,500 liters of air in 1 hour, making 1 gm. of starch
    - 4. changes following the photosynthesis of sugar
      - (1) change of sugar to starch—often implied in term photosynthesis; but light not necessary for this change (e.g., starch in underground stems and roots)
        - —accomplished by common hydrolytic process — removal of H$_2$O
        - —regulates per cent of sugar in solution in cell
        - —advantage in change of sugar to starch: starch

does not interfere with osmotic pressure of cell, stores more compactly, ferments less readily.

- (2) change of simple sugars to double (as cane sugar)
- (3) change of starch back to sugar
  - — reverse of (1) above; also a common hydrolytic process
  - — necessary before starch can leave the cell
  - — disappearance of starch from leaf cells at night thus explained.
  - — change of this transfer sugar to storage starch in seeds, roots, etc.

- c. spongy tissue
  - (a) between palisade tissue and lower epidermis
  - (b) loosely-packed, with irregular spaces throughout, allowing rapid changes in the gaseous and vapor content
  - (c) relatively so placed as to give firmness (at least when turgid) through the "braced" positions of the cells
  - (d) functions
    - 1. photosynthesis and related processes as in palisade tissue
    - 2. protein manufacture using nitrates, etc., from the soil water
- d. fibro-vascular tissue
  - (a) supporting tissue
  - (b) conducting tissue
    - 1. water (xylem) — in perennial, woody stems acting finally as supporting cells — the *wood*
    - 2. food (phloem) — in woody stems pushed to the outside forming the *bark*

(C) Respiration, a function common to all leaf tissues

- a. process common to all living cells, plant and animal
- b. sources of oxygen
  - (a) general air supply
  - (b) oxygen liberated in photosynthesis
- c. oxygen as source of energy
  - (a) necessary
    - 1. special work of cells (photosynthesis, absorption, etc.)
    - 2. digestion of food itself
    - 3. growth and repair of cells; assimilation of substances
    - 4. reproduction (increase in number of cells and in cell substance)
  - (b) eliminated mainly as H_2O and CO_2
  - (c) amount necessary for plants small; resulting CO_2 small
    - 1. plants less active than animals
    - 2. little CO_2 given out by cut flowers or by green plants even at night; (popular belief: plants injurious in sickroom, etc., due to heavy odor, not to CO_2 and O relationship).

C. Comparison of CO_2 and O in relation to green plants and animals

	Carbon dioxide	**Oxygen**
(A)	Raw material used in photosynthesis	Necessary for metabolic processes of living cells
(B)	Liberated by green plants constantly, day and night	Liberated by green plants during the process of photosynthesis, during daylight only
(C)	Amount liberated small	Amount liberated large, completely masking CO_2 elimination in day time, leading to popular misconception: "Plants breath out CO_2." Excess O available for animal consumption
(D)	Of no further use to non-green plants or to animals	Necessary for all animals and all plants

(E) Plant Respiration and Photosynthesis cycle for O and CO_2 (diagram)

(F) Animal respiration duplicates the lower half of this cycle, providing the CO_2 necessary for, and using the excess O resulting from, photosynthesis, as shown in the diagram

D. The Green Plant an *Independent* or self-sufficient Organism

(A) Can complete full life cycle using only *inorganic* materials as source of food and energy; animals and non-green plants must have as such sources food substances in *organic* form. All sugar, starch, protein, and fatty foods are traceable ultimately to plant origin.

(B) Supplies in photosynthesis the oxygen for its own metabolic processes; while animals and non-green plants depend upon green plants for their oxygen. Green plants use in photosynthesis a waste (CO_2) formed in respiration; in respiration, in turn, green plants utilize part of the waste (O) formed in photosynthesis.

(C) Has a water rotation, as illustrated by Wardian cases or closed fern globes. Compare the dependence of animals upon plants with regard to water (rain fall (amount and distribution) and humidity).

STRUCTURE OF THE LEAF

1. Cross section of a leaf (magnified 60×). Stained: the green chloroplasts appear as purple grains or disks. Compare this microscopic view with the accompanying illustration, and identify the various leaf tissues: upper and lower epidermis, palisade tissue, and the looser, spongy tissue below. Locate at least one stomate in the epidermis.

2. A fresh, unstained cross section of a leaf. Note that the chlorophyll grains are really green here.

3. Leaf section showing a stomate more clearly. Note the two smaller cells between which the opening lies. These cells are called guard cells, and they regulate (to some extent) the size of the opening and, consequently, the amount of moisture passing out. (Water does not normally pass into a leaf through the stomates.)

4. Lower epidermis of a leaf, stripped off, and placed flat upon the glass slide. This is, therefore, a face or surface view of the lower side of a leaf. Note that the general epidermal cells are separated by the paired guard cells. Between these crescent shaped cells is the opening through which an exchange of gases is effected, (chiefly CO_2 and O) and through which H_2O passes out.

5. Cross section of a leaf showing (1) a vein cut across, and also one cut lengthwise. Find at least one tube with ringed or spiral thickenings as pictured in the accompanying illustration.

6. Petiole of a leaf cut lengthwise. Note that the vein tissue is continued through the petiole, connecting the blade with the stem tissues. Identify the ringed water tubes. Can you find any other tissues named in the accompanying illustration?

7. This celery leaf stalk was placed in the glass of colored water (eosin or

red ink) at the beginning of the period. Cut across with a clean knife and note that the colored liquid has traveled up in special regions, the veins.

8. A leaf picked up in the woods in early spring. The complete network formed by the veins is very evident here. Which tissues have rotted away?

PHYSIOLOGY OF THE LEAF

Transpiration Experiment. One or two leaves are here inserted in a cardboard disk and the ends placed into a glass of water, the disk resting on the top of the glass. A clean dry glass is inverted over the leaves, the open end resting on the cardboard. Examine the glass inverted over the leaf in 24 hours or less. What change do you note? Does the control part of the experiment (differing only in lacking the leaves) show the same change? Why? Is the control part of this experiment necessary?

Write up this as an experiment, dividing your work into at least 4 main parts. (1) Aim or question to be solved; (2) procedure; (3) results; (4) conclusion or interpretation. In writing up experiments, a fifth part, applications, is possible and often advisable. These are so far reaching that a separate page might well be added for them as they are found; it is, of course, not possible to realize all of them at the time of the experiment.

Starch in Leaves

1. Starch and water and iodine give a characteristic color reaction when combined. The color varies from light blue to black, depending upon the proportions used (as you may demonstrate with the above-named substances in this tray).

2. Test (if new to you) each of the substances in the accompanying dish for starch: cracker, while potato, sweet potato, bean seed.

3. Our text-books tell us that leaves manufacture starch. How can we determine this? To see any color reaction it will be necessary to remove the chlorophyll or green coloring matter first. This is soluble in alcohol, and can be extracted by soaking the leaves in alcohol 6 to 24 hours. (Replace with fresh alcohol, if necessary. The process can also be hastened by first boiling the leaves in water (3-5 minutes) and then decolorizing in alcohol.) The leaves will finally appear whitish or yellowish white.

4. Nasturtium leaf (from a sunny garden) picked at 4 P. M., decolorized, and treated with iodine. Does the leaf contain starch?

5. Leaf from the same plant picked at 6 A. M. (before any sunlight reached it) and treated as above. Why does this not give the starch reaction? What becomes of the starch formed each day? This leaf picked at 6 A. M. shows that it does not remain in the leaf. A small part is doubtless used by the leaf cells themselves. Most of the starch leaves the leaf during the night. To do this (see osmosis later) it has to change back to sugar. Finally it may against be changed to starch for storage in the stem (white potato), root (sweet potato), fruits (banana), or seeds (bean).

6. Part of this leaf was covered by a bit of tin foil or cork; the rest of the leaf was then left exposed to the sun as usual. Explain the position of starch in this leaf. What is *one* essential of starch formation?†

7. A white-edged geranium leaf was picked at 4 P. M., decolorized, and treated with iodine. Which region contains starch? What is a second essential for starch formation?

8. Half of this leaf was covered with vaseline to prevent access of CO_2 through either surface. What is a third essential for starch formation?

9. A red coleus (Iresine) leaf tested for starch. This shows that chlorophyll may be present and active, though masked by a second coloring matter.

10. Starch (including sugar) formation by leaves has been fully covered by the lecture and the present demonstration. The sunlight requirements, the time for

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†Sufficiently definite for this general introduction to plant cell activities, particularly since most text books (by implication, at least) include starch formation under photosynthesis.

decolorizing, etc., make it a tedious experiment for classes with as irregular hours as ours. Any student to whom this is wholly new may have materials for individually demonstrating this function of leaves.

11. Write up one of the experiments dealing with starch in leaves. It may be simple demonstration of the presence of starch in green leaves, or a more complicated one, bringing in the necessity for one of various essentials, CO_2, sunlight, or chrolophyll.

Respiration Experiment

Preliminary statement. To demonstrate respiration at least one of the two phases of the process must be proven — either the exhaustion of O from the surrounding medium, or the addition of CO_2 to it. The latter is much easier to do, especially with *green* plants. (Recall the oxygen liberated in photosynthesis.)

To a glass of lime water ($Ca\ OH$) or barium water ($Ba\ OH$) add CO_2; (run in a little charged water containing CO_2; blow your breath into the glass; or simply leave the glass of lime water exposed to the air of the room with its contained CO_2). The carbon dioxide unites with the lime water, making a whitish substance, Calcium carbonate ($Ca\ CO_3$), which may remain for a time on the surface as a definite film, cloud the liquid, or settle to the bottom as a friable precipitate.

How can we use this knowledge to demonstrate that respiration is a function of green plants?

Experiment. Be prepared at the next class session with a suggestion for experimental demonstration of green plant respiration.* (Be sure that you take the CO_2 already in the air into consideration in planning your experiment.)

How much difference between the leaf-enclosed limewater and the control limewater? To what is this *difference* due? Does possible leakage of CO_2 (in or out) affect the apparent difference? Does the time affect the results obtained?

Write up this experiment very carefully, using the directions already given; hand it in one week from to-day.

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*Set up much as described in *Torreya* (Dec., 1911, p. 261), or in Bigelow's Teachers' Manual of Biology (p. 35, Macmillan).

II. CELLS

Illustrative material:
  - Turgid and wilted seedlings
  - Leaf and stem charts showing cell structure
  - Charts illustrating cell types and parts; plastids, cell crystals, starch and protein granules, etc.; composition of cells (water, ash, etc.)
  - Chart showing mitotic changes in nucleus

A. Types already seen (green plant: leaf, stem, root) differing in (1) size, (2) shape, (3) presence or absence of cell contents (living or non-living cells), (4) parts visible, (5) color (natural or due to affinity for various stains).

B. Cell parts
  - (A) Parts common (to live cells): (1) wall, (2) cytoplasmic membranes, (3) cytoplasm, (4) nucleus (and one or more nucleoli), (5) one or more vacuoles
  - (B) Comparison of plant and animal cells, especially with regard to cell wall and vacuole

C. Morphology and function of these fundamental parts
  - (A) Wall
    - a. support
    - b. modifications — increased thickness, effect upon absorption and excretion; upon life of cell itself

    - c. type of substance deposited, (lignin, chitin, wax, slime)
    - d. adaptation for both absorption and support, (thin areas in thickened water tubes)
    - e. not sole method of support; compare cell turgidity (seedlings, young leaves)
  - (B) Nucleus
    - a. initiates cell division
      - (a) relation between rate of division and number of nuclei in a cell
      - (b) nuclear changes in ordinary cell division
    - b. important in control of cell activities
      - (a) recent experiments with de-nucleated cells
  - (C) Cytoplasm or cell body, also active part of cell
    - a. absorption, excretion, enzyme activities, and other metabolic processes
    - b. visual evidences in some cells — Amoeba, Elodea, muscle, and gland cells
    - c. theories concerning structure; elements present
  - (D) Cytoplasmic membrane
    - a. position — bounding cytoplasm, separating it from all other parts, as vacuole, wall, nucleus; often invisible in normal cells
    - b. selective power
      - (a) due to semi-permeable character
      - (b) not wholly helpful
      - (c) recent discussion of cytoplasmic membrane and its permeability
    - c. relation to cell turgidity; compare osmosis experiments on passage of substances into and from the cell
  - (E) Vacuole
    - a. false implication of name
    - b. relation (size) to age of cell
    - c. storage of useless and of harmful substances, as salts (crystals)
    - d. influence of salts, etc., on absorption; on cell turgidity
    - e. ready water reservoir
      - (a) hydrolytic changes
      - (b) other metabolic processes (water eliminated may be many times water obtained from food, etc.)
    - f. coloring when present — often responsible for blue, red-blue and blue-purple colors; indicator (like litmus) in iris, hydrangea, hepatica, etc.
- D. Other cell parts — not common to all plant cells
  - (A) Plastids
    - a. chloroplasts (already discussed)
    - b. leucoplasts
      - (a) relation to chloroplasts
      - (b) starch storage
    - c. chromoplasts
      - (a) lipoids?
      - (b) crystalizing in varied shapes
      - (c) present in yellow-red, yellow, and orange flowers, fruits, etc.
  - (B) Crystals
  - (C) Food grains, other than plastids — mainly protein or glycogen (see demonstration)
- E. Comparison of cell activities
  - (A) General
    - a. common to all plant cells — even the simplest or smallest
    - b. numerous; a partial list includes:

(a) absorption of water and minerals in solution
(b) absorption of foods (also in solution)
(c) oxidation and splitting of foods to obtain energy, heat, building materials
(d) synthesis of above substances into stored foods, cell substances
(e) formation of special substances: crystals, pigments, enzymes
(f) growth and repair with all implied assimilation changes
(g) reproduction; cell division

(B) Special examples: absorption, root-hair cells; photosynthesis (chlorophyll-bearing cells); transfer of water (non-living tubular cells), etc. Division of labor indicates cell's contribution to life of other cells of same plant; refers to its special activities, may not lessen markedly general activities listed above

(C) Chemical and physical changes in above processes astoundingly complicated; how many can be duplicated or imitated in the laboratory?

F. Common mistakes in concepts of cell

(A) Two instead of three planes; corrected only by proper handling of microscope

(B) The cell as morphological rather than living, physiological unit; latter character probably more important

(C) Confusion of cell *parts* with cells as a whole; a large majority of students and teachers mistakenly speak of the "wall" and "nucleus" of a starch grain; or of the "cell division" of a chloroplast.

(D) Such over-emphasis of the various component cell substances (chromatin, lignin, etc.) that a plant cell fails to be comprehended as a protein unit, with a high percentage of water (in the cytoplasm and vacuole) surrounded by a (practically) carbohydrate envelope or cell wall, and containing more or less stored food substance (sugars, starches, fats, and proteins).

LABORATORY STUDY OF THE CELL

1. **Introductory exercises with the microscope.** Study a cross section of a leaf with both high and low powers. Draw enough to show all the types of cells present, labelling each type. This drawing need not form part of the completed note-book, as this exercise is inserted mainly that the student may demonstate her ability to use the microscope and to interpret correctly what she sees.

2. **Study of a cell with well-defined parts.** Pull off a bit of onion epidermis from one of the inner scales of a good firm onion. Mount directly in water. Lower the cover-glass slowly and obliquely to avoid including air bubbles. Examine (low and high power). (Why is a small piece better for this work than a large piece?) Draw iodine-eosin under the cover-glass with a strip of filter paper (as shown). Draw (on unlined paper) 3-4 adjoining cells, making each cell at least one inch long. Label each cell part: cell wall, cytoplasm (cell body) and nucleus. Avoid the cells undergoing disintegration, which show a disorganization of the nucleus. Focus carefully on the nucleus; one or more definite bodies may be distinguished (nucleoli). These are important, in this exercise, not so much from the point of cell structure, as to insure that you are using the microscope with sufficient skill. Practice with the fine-adjustment screw until you see its value.

3. **Plasmolysis.** To the stained onion mount add 10% salt solution. (Draw it under as you did the eosin.) Select several cells with definite nuclei and watch

them for changes. Describe. What cell part hitherto unnoticed now becomes evident? Are the cells undergoing disintegration affected in the same manner? Explain. Replace the salt solution with water. Do any of the plasmolyzed cells recover? All? Explain.

4. **Additional exercises* with cells and starch grains.** (a) Cut or scrape a thin slice or section of white potato. (This slice may be cut with a sharp razor (freehand); it is easier to get thin sections if a hand microtome is used; or scraping a cut surface with a sharp knife will usually yield a piece suitable for study.) Rinse in water and mount for study. After examination (low and high power) draw a little iodine under the cover-glass. Draw two or three adjoining cells, showing the cell parts that are visible. Do you find the cytoplasm? nucleus? Explain. (This and the following exercises are mainly for interpretation of cell structure; but they are also an aid in forming a fair relative idea of the sizes of ordinary plant cells, their contents, smaller cells (yeast) and, finally, bacterial cells.)

***Exercises (c) to (f) are included in the next demonstration. Exercise (f) may also be completed then, if you cannot finish these exercises during this period.**

(b) Potato starch grains. Draw 2-4 starch grains (high power). Stain *slightly* (so that the markings are not obscured).

(c) Compare the white and sweet potato (thin sections).

(d) How do corn starch grains differ from the above starch grains? Wheat starch? Are all starch grains alike? How could the microscope be used to detect many adulterations of food, drugs, etc.? Look at the accompanying microscopic picture of various plant cells (e.g., wheat starch) in this sample purchased as black pepper.

(e) Mount and stain (with iodine) a little compressed yeast. What kind of starch is used in these yeast cakes?

(f) Crush the potato starch by pressing upon the cover-glass; examine. How may the grinding of foods affect the starch grains? The availability of the contained starch?

DEMONSTRATION: CELLS AND CELL PARTS

Usual cell parts

1. Cross out of a tulip bud (×60) showing petals, etc. Identify in these cells the parts already studied in the onion epidermal cells: cell wall, cytoplasm, nucleus. Where is the cytoplasmic membrane? Is the vacuole evident? In making sections, we cut through as well as between the cells. Explain the apparently empty cells in this slide.

2. Onion epidermal cells stained with iodine-eosin. Examine the nuclei until you find one which shows one or more nucleoli. (If you are not able to pick these out readily, you need more practice with the microscope. Arrange for it this week.)

Cell movement

3. When cells contain chlorophyll grains, the grains are sometimes carried along by the moving cytoplasm. In such cells one can judge better of its rate and the time necessary for a complete circuit of the cell. The water plant, Elodea, has very thin leaves, one layer of cells only, except near the midrib. One leaf has been broken from the plant and placed under the microscope (×60). Note the brick-shaped cells with their tiny chlorophyll grains. The second microscope shows the same thing (×200). Watch until you see chlorophyll grains carried around the cell by the active cytoplasm. The movement is often best at the edges of the cell (because the interior of each cell is usually one large vacuole). Watch one chlorophyll grain and find how long it takes to make a complete circuit. Is the rate of movement affected by the magnification of the microscope? Explain. Is the time for one circuit affected? Explain.

Stem cells

4. Cross cut of a fern stem. Move the slide freely; how many types of cells (size, shape, thickness of walls, presence or absence of contents, and coloring*) do you find?

5. Fern stem. Near the center of this stem are (brown) cells so thick-walled that they are practically solid. How would this affect the transfer of materials into and out of such cells? Certain thick-walled cells lose their contents and become water conductors; others serve for support, giving rigidity to the stem.

6. Long cut through a fern stem showing the tubes that carry food and water up and down the stem. The water tubes are thickened or ridged much as shown in the accompanying illustration.

7. Cross cut of a fern stem showing (yellow) water tubes, (brown) supporting cells or fibers, and (almost colorless) food tubes. The main part of the slide is filled with slightly-differentiated packing or parenchyma cells.

8. Cross cut of a pumpkin stem, showing nine darker spots (×40). Each of these spots ("bundles") is a collection of tubes that carry food and water. How many types of cells present in this stem?

9. Cross cut of a buttercup stem, showing six distinct bundles of food and water tubes. In this section the parenchyma (globular, thin-walled cells) is much more prominent than in any of the preceding.

10. A single bundle of food and water cells (×200) with some thick-walled supporting cells. How many show contents? Explain.

11. Pine stem (×80) cut lengthwise through the water tubes so as to show rounded areas in the walls of the cells. These are peculiar thin regions, allowing rapid cross transfer (water and substances in solution) without really interfering with the main vertical flow of the water.

Root.

12. The root cells that differ most from the stem cells already seen are the root hairs. These corn seedlings (germinated on wet, dark blotting paper between two plates or saucers) bear numerous root hairs, tiny elongated cells with thin walls, which absorb water rapidly. Examine with a lens, and under the next microscope (×60); and compare with the illustrations and their legends. Note the apparent lack of cytoplasm under the high power. Explain. Why are such large water reservoirs an advantage? (Recall the structure of these cells when studying molds.)

Special cell bodies: plastids and food granules

13. In the Elodea you saw chloroplasts of the usual type. In the cylindrical cells of this thread-like plant (Spirogyra) are curious ribbon-shaped plastids twisted around inside the cell. On these bands distinguish little dark dots, as illustrated in the accompanying picture; these dots indicate centers of starch formation. (Near the center of each cell is the nucleus, partly hidden by the winding chloroplasts. It has a distinct nucleolus (see illustration), and can be easily distinguished from the starch centers in the choloroplasts.)

14. White potato (stained with iodine, as are all the following starch slides) (×60), showing loose globular cells, some of which are still filled with starch grains. About how many to a cell? Why is it incorrect to say "starch cells"?

15. Sweet potato; how do the starch grains differ from those of the white potato? One often hears, "I can eat sweet potatoes, but not white, as starches are forbidden." Is the implication justified by this slide?

16. Cornstarch. In the center you will usually find one or more cracks. How does the margin differ from the starch grains already seen? Are concentric markings visible?

17. Flour. This contains many broken bits of the plant cells as well as the starch grains. Note the value of iodine in distinguishing between them. How do these differ from the starch grains previously seen (2)?

18. Starch grains of potato — partly crushed as by grinding of foods. The outer envelope of some starch grains is composed of cellulose; others, of starch. Relate this to properly chewing food; to grinding foods.

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*Sections are stained with mixed stains, or a series of stains. The various cells differ in their power to take and to retain stains. All parts of each slide are subjected to the same stains; the fact that some cells finally appear yellow, while others in the same section are brown or red or green, shows that they are really different kinds of cells.

19. Cooked potato starch, afterward stained with iodine. Can you distinguish the envelopes of the starch grains? What is gained by cooking starchy foods?

20. A bit of Uneeda biscuit, mounted in water, and stained with iodine. Was it properly cooked? Bring some bread from home, and test that for yourself, if you care to do so.

21. Chromoplasts. A thin slice of carrot (×200) showing tiny indefinite bodies in the cells in and near the rind. Similar bodies occur in most red, yellow, and orange flowers and fruits (nasturtium, rose, etc.).

22. Protein granules. A section of fern stem, showing protein granules in the parenchyma cells. In the next microscope (×200) focus with care to bring out the concentric markings.

23. Part of a wheat grain, sectioned to show the inner starch-bearing cells (unstained) and the outer cells with protein granules. (The latter are stained blue; do not, therefore, confuse them with starch grains which you are accustomed to see stained blue.)

Other cell studies.

24. Cross cut of a trillium stem stained pink (×80). In this part of the stem distinguish two kinds of cells — (1) with granular protoplasmic contents and a deeper stained (red) nucleus; (2) cells of the same size and shape lacking the parts seen in (1), but nearly filled by a mass of needle-like crystals. Recall the contents of the potato cells. Explain the lack of cytoplasm and nuclei in these crystal-containing cells.

25. Vacuole: A bit of onion root (×80) placed so as to leave young cells near the center of the field of observation, and older cells near the right edge of the field. With age, what changes are noticed in the vacuole? in the relation of the nucleus to the size of the cell?

26. Cell division: The simple method of cell division obtaining commonly in yeast and bacteria differs greatly from the common method of cell division (mitosis). Unless familiar to you, read the description of this process in the accompanying book (Curtis), and identify in this bit of hyacinth root at least three of the stages illustrated there.

III. OSMOSIS AND DIALYSIS

1. a. A glass tube was partly filled with molasses and then a piece of soaked bean seed-coat (Lima bean or better the Broad Windsor bean) was tied tightly over the bottom end. The upper open end of the glass tube allows room for expansion or increase of the contained liquid. The molasses was level with the gummed label at the hour indicated yesterday. Note its present level. The attraction between such substances as sugar and water is too well known to require restatement here. What effect did the molasses (sugar) inside have upon the water? How do you know that water passes into the tube? Did the attraction between water and molasses cause any of the molasses to pass out? What will be the effect of changing the water in the outside jar?

1. b. Compare the results in this second tube where a strong colored salt solution (e.g., copper sulphate) was placed in the tube.

2. The tube may be compared to a cell and the bit of seed-coat may be viewed as part of the cytoplasmic membrane (and cell wall); the molasses to the cytoplasm and the substances (salts, sugars) in solution in the cells. When water passes through this membrane into the molasses, it is evident that if the tube were closed at both ends, the increase in volume in the tube would increase the internal pressure or turgidity, just as the turgidity of cells is increased.

3. This second artificial cell was originally filled with *water* and immersed in *molasses*. What changes have occurred? This is put up to make sure that a false impression is not given; i.e., that water is always passing *in* and not *out;* and that substances in solution pass *out* and never *in*. In a living cell many substances, foods and other chemical substances, may be passing through its cell

membranes at the same time. It is also true that one cell may be taking in water from the soil and at the same time passing water out to an adjoining cell; or a cell may be absorbing a sugar or a salt from one cell while giving that and other substances to a third cell.

4. Similar potato (or beet) slices soaked in plain water and in strong salt solution.

Feel of these pieces; bend slightly. Explain the difference in rigidity by explaining what has happened to the cells themselves. If you examined a thin slice of the salt water potato under the microscope, how would the cells compare with the plasmolyzed onion cells?

5. Read pages 74-83 of Atkinson's First Studies of Plant Life for a simple but complete application of these changes to plant cell activities.

6. Raisins soaked in plain water and in strong sugar solution. Which are plumper? In what direction was the water current in the water-soaked raisins? In the sugar-solution ones?

Though each raisin *is* composed of many cells, a raisin might be used to illustrate the activities in a single cell, the skin being interpreted as the cell membranes (cytoplasmic membrane and cell wall). (Parchment and other membranes must usually be obtained through a special supply house: if left in solutions, they are easily injured by the action of bacteria, etc., developing in the liquids used. Raisins or prunes with unbroken skins are inexpensive and easily procured).

7. Molecules of water, sugars, salts, etc., differ in size. Explain, therefore, why the volume on the salt or sugar side of a membrane might rapidly become greater or higher than that on the water side. How long would it remain so?

8. If cane sugar, which has an attraction for water, but cannot pass through most membranes, were used instead of salt, it would continue to attract the water, without itself passing through the membrane. What effect would such substances have upon the water content of a cell containing them? How would a large amount of such substances affect the turgidity of a cell?

9. Water (or food substances) may be transferred from the cell absorbing or manufacturing them to the next cell, if it is lower in its per cent of that substance. For example, in the diagram accompanying this sheet, cell 1 is a surface cell on a root. (If a root hair, it would be extended as shown by the dotted lines, increasing its absorbing surface enormously.) If cell 1 absorbs a salt (calcium nitrate, for instance) from the soil, it will probably have more salt in proportion to its water than cell 2. Cell 1 will transfer some of its calcium nitrate to 2 (and 2 will probably give a little of its water to 1). Cell 2 will then differ from 3 as 1 differed from 2, and a similar process will take place between 2 and 3, 3 and 4, and so on until a water tube is reached, which will carry the calcium nitrate solutions on up the stem toward the leaves. Work out a similar explanation of the changes in the water content of a series of leaf cells lying between a water tube and the epidermis, where evaporation of water may take place.

10. The interior of this carrot was hollowed out and filled with sugar (or molasses). The top was then plugged with a cork having a glass tube in it, as in the first experiment. The carrot was immersed in clean water. What change will take place in the molasses cavity? Will the water surrounding the carrot gain molasses? Explain.

11. Explain the shriveled appearance of these berries (fruits, leaves or seeds may be substituted). What have they lost? What change has taken place in each cell? The loss of turgidity means collapse of the cell, and shows in the consequent wilting of a plant, wrinkling of the leaves, fruit, etc. This loss of water is very important in cell metabolism. When a cell loses its turgidity, becoming plasmolyzed, the cytoplasmic membrane no longer occupies its normal position against the cell wall. Such cells do not absorb or transfer substances normally. Besides, the loss of water may mean too high a per cent of acids, etc., in the cell. If plasmolysis is too extreme, the death of the cell follows. Did your plasmolyzed onion cells recover? Does the time that elapses before wilted flowers are put into water affect their recovery?

12. Note that both the process and applications of osmosis and dialysis have been fully illustrated, without using either term, or any other technical terms, such as hypertonic solution, ionization, osmotic pressure.

In the latest dictionaries you will find osmosis defined as including passage (1) into and out of the cell; and (2) the passage of water only or of substances in solution, such as sugars and salts.

The direction (in or out) is not emphasized now by most people. Many textbooks, however, distinguish between the passage of water and the passage of substances in solution (salts, sugars, etc.), calling the passage of water through a membarne osmosis, and the passage of the sugars, salts, etc., *dialysis*. (Others define osmosis as the passage of the *solvent* (usually water, of course); and dialysis, as the passage of the solute (sugars, salts, etc.).

Whatever terminology is used, the following introductory statements will be of value: 1. Substances in solution tend to distribute themselves equally. (It is never necessary to shake a solution before using; mixtures (including most medicines) need to be shaken, of course.) 2. This equal distribution of one (or more) substances throughout another is called diffusion. 3. When this diffusion is hindered by a membrane (as in the various experiments above and in living cells) we call the process osmosis or dialysis (as discussed above). 4. The membrane affects this exchange in two ways:

(1) It is slower.

(2) It is more or less "selective," depending upon the size of the molecules of the various substances when compared with the ultra-microscopic pores of the membrane.

13. These texts (Halliburton and Howell, etc.; see respective indexes) give a more complete description of these processes and the factors affecting them. If interested, read one of these authors on this subject.

IV. MOLDS

Illustrative Material:

Charts showing molds (life histories and variations in structure);
Preserved specimens showing mycelium and spore-bearing parts: tree-fungi, edible mushrooms, puff-balls;
Fresh fruiting specimens (on bread and on agar) of Mucor, Pencillium, and (red) Monilia;
Selected illustrations of types of molds from various college text-books

A. Molds as plants
  (A) Position of molds in plant classifications
    a. relation of molds to other micro-organisms included in the course: yeasts and bacteria
  (B) Fungi as degenerate plants
    a. simple structure
      (a) few types of cells
      (b) these but slightly differentiated
      (c) chlorophyll lacking
      (d) methods of reproduction
B. Life history of molds
  (A) Mucor (having a many-spored sporangium)
  (B) Penicillium or Monilia (bearing conidia)
C. Comparison of molds
  (A) Of the molds studied in this course
  (B) Of those with other molds
    a. puff-ball, edible mushrooms
    b. molds having alternate hosts, as barberry rust
  (C) Characters common to all molds
    a. chlorophyll lacking
    b. vacuoles prominent with age
    c. usually two definite parts: mycelium and spore-bearing parts

- (D) Main ways in which molds differ
  - a. mycelium — septate or non-septate structure
  - b. reproductive bodies — types (not limited to single kind in many species)
  - c. hosts or substrata
- D. Prominent cell characters of molds
  - (A) Thin mycelial walls
    - a. demonstrate quick collapse
    - b. relate to normal mold habitat
      - (a) water or moist places
      - (b) stagnant air
      - (c) unsunned spaces
  - (B) Large amount of water
    - a. vacuoles very large — especially with age
    - b. rapid absorption of water
      - (a) swelling confused with actual growth
      - (b) explains relation to humidity, rainy periods
    - c. total amount often 11/12 to 19/20 of weight
      - — common mis-statement concerning food value (% of protein)
  - (C) Nuclei
    - a. not visible with ordinary stains
    - b. cells often multi-nucleated
- E. Comparison with ordinary green plants
  - (A) Molds lack chlorophyll; must have some of their food in *organic* form
  - (B) Cell substances otherwise as numerous and as complicated as any non-green cells:
    - a. numerous proteins and albuminoids
    - b. glucose, glycogen, gums, oils, resins, cellulose, chitin
    - c. organic acids, pigments, alkaloids (muscarin, ergot)
  - (C) Other more striking differences: size, habitats, length of life cycle, seeds versus spores, complexity of structure (number, types, and specialization of cells)
- F. Species of fungi
  - (A) Number
    - a. over 15,000 in a recent classification
    - b. vary with locality (temperature, hosts, etc.)
  - (B) Helpful to man
    - a. general decay organisms
    - b. as foods: mushrooms, cheeses
    - c. as medicines (ergot)
  - (C) Destructive to foods: to foods and to food-producing plants
  - (D) Pathogenic molds
    - a. man: ring worm, favus, thrush, less often, abscesses, tumors
    - b. other animals
      - — cow "lumpy jaw"; lung infections
      - — fish and insect diseases
- G. Control of injurious forms (see effects of physical and chemical agents later)
  - — limited by necessity of using agents that will not irritate or destroy the host tissues, spoil food substances, etc.

LABORATORY WORK ON MOLDS

1. Place a sterile cracker (as shown) in one of the covered watch-glasses. Moisten this cracker with sterile water from one of the cotton-plugged test tubes. (Too much water favors bacterial rather than mold growth.) Then inoculate (with sterile forceps) the moist cracker with mold selected from one of the dishes containing bread covered with various molds. Date; label to indicate the mold selected. Place in what you consider a favorable place; and later explain the results obtained with reference to (1) type of mold obtained, (2) evident purity of the culture, and (3) the relation of water, light, and temperature to the rate of mold growth. Keep the culture 10 days. If but one type of mold develops, place it in the tray provided for pure cultures; if two or more species are found, inoculate a fresh cracker from this or another mixed culture to secure a pure culture (so far as molds are concerned).

2. Examine your dishes of mold with a hand lens and describe the general cobwebby growth or mycelium. Note the large bulk compared with the amount of food apparently used. Explain.

3. Study the common black or bread mold growing on the inside of the inverted porcelain dishes. Pick up one cluster with forceps and study with the low and high powers. (Avoid crushing; disregard bubbles.) Draw what seems to be one plant, labelling all parts. Can we say that molds have true roots? stems?

4. Compare young and old mycelial threads in color and contents. Account for the differences. Do you find any indication of cell structure? Find young and old spore cases. What changes occur with age? After the blackboard discussion of the formation of these spore cases and their development, construct a series of drawings or diagrams from your slides and those belonging to other members of the class, showing as many of the spore-case stages as you can find.

5. Remove the cover from the very fluffy growth on the slice of bread; compare its present appearance with that observed at the end of the period. Explain.

6. Add a few mold spores to the dish of sugar solution (1%, 3%, 5%, 10%) in which you would expect them to grow best. Examine 1-3 days later to see if you selected a favorable percentage.

7. Study the red mold (or blue mold) as above. Mount and examine bits of one of these molds, until you can construct a representative mold plant: mycelium and spore-bearing part. Compare with the bread mold, *listing* all the likenesses and differences.

8. Use the key in Buchanan to trace one of the above molds. Which characters (likenesses or differences) occur most often in this key? Why?

MOLD DEMONSTRATION

1. This twig bears an orange-colored mushroom, in which the colored mycelium can be traced back into the rotting wood. The spores are borne on the outer, roughish surface of the orange-colored fruiting body.

2. This jar shows various stages in the development of the common mushroom: mycelium to mature spore-bearing mushrooms. Note how little of the hidden mycelium adheres to a picked mushroom. The spore-bearing part is, nevertheless, always dependent upon a cobwebby mycelium which penetrates a substratum such as decaying leaves, humus soil, rotting wood, thus securing food and moisture. The little knobs or buttons here and there on the mycelium develop into the parts commonly called mushrooms, (toadstools, puffballs, etc.). On the

under side of the umbrella-like top the spores are produced — commonly all over the surface of the hanging leaves or gills (or in other species in the little holes or pores). The spore color varies with age, and with the species (white, pink, brown, yellow, etc.).

3. Barberry leaves with mold spots. A section through one of these leaves is shown in the next microscope. It is a little cup-shaped cavity filled with strings of black spores (conidia). In the next chart you can trace their connection with the mycelium crowded between the leaf cells. On wheat these spores develop into a different kind of mold plant, producing very different types of spores (See illustration). These in turn grow most readily on the barberry. Such alternation in hosts is not uncommon among microorganisms, especially among the protozoa (e.g., malaria). (See Plant Diseases later.)

4. Molds growing on human tissue, skin, hair, etc., are pictured here. Exchanging hats or using public combs and brushes may be attended with real dangers.

5. A bit of tissue (lung of cow) showing the threads of a mold growing within it. Find an area where the lung tissue has been disintegrated.

6. Microscopic slides showing two types of spore production among the molds: spores produced singly (one or a series) at the ends of mycelial threads, or many spores produced in a common sac.

7. Bread mold showing characteristic runners. How do the little mold plants upon the sides of the *dish* get their food and water?

8. A septate mold and a non-septate mold are shown in this and the next microscope. The mycelium of this mold is non-septate or almost so, while the other is quite fully septate. Do the septa have any relation to the branching? to the type of spore production?

9. Mold threads stained to bring out the nuclear masses. Notice that each apparent cell contains more than one nucleus.

10. A series of slides to show the gradual development of the spore case in bread mold. Note the changes in color and in the density of the mycelial and spore case contents; add any not previously seen to your own series showing the development of a spore case.

11. Germinating mold spores, from the 2 and 3% sugar solutions. Is there any reason why they should be more numerous in these solutions than in the weaker or in the stronger solutions?

12. A 10% salt solution left from last year. What is there surprising in finding molds in this? Explain why pickles do not always "keep"; why mackerel in brine may grow "rusty."

V. YEAST

Illustrative material: Charts (1) bread yeast; and (2) peach curl (or other mycelial yeast).

A. Life history — of a mycelial yeast (peach curl)
  — of bread yeast
B. Yeast a mold
  (A) Reduction of mycelium — in commercial yeasts
  (B) Methods of reproduction
    a. characteristic "budding"
    b. spore production: conidia; sporangia (few-spored)
  (C) Cell structure
    a. parts present
    b. chlorophyll lacking
    c. nucleus and staining properties (distinguish from crystals and glycogen granules)
    d. vacuole — large size
C. Utilization of food materials
  (A) Protein — necessary?
    — compare molasses and sugar as foods

- (B) Carbohydrates
  - a. starch (flour in bread making)
    - (a) diastase enzymes
      - 1. necessary
      - 2. supplied (1) cells of wheat, barley, etc.
        (2) yeasts?
  - b. sugars — two types of changes due to yeast enzymes
    - (a) first type: double sugar to simple sugars
      - 1. work outside of the yeast cells (simple sugars may then pass into cell)
      - 2. hydrolytic (as in starch)
        - (1) saccharose (sucrose) by invertase (sucrase)
        - (2) maltose by maltase
    - (b) second type: simple sugars to CO_2 and alcohol
      - 1. work done inside yeast cells (heat and energy thus liberated available)

- D. Characteristic changes accompanying the use of food materials
  - (A) Fermentation
    - a. origin of word
    - b. various implications in usage of term
      - (a) carbohydrates not proteins
      - (b) carbohydrate changes yielding gas (acid not considered); gas zero not sharp
      - (c) carbohydrates or proteins, yielding gas, or acid, or both
- E. Conditions affecting growth
  - (A) As in other molds
  - (B) Temperature
    - a. optimum 23-40° C.
    - b. effects of extremes outside this range
    - c. apply (a) to methods of keeping various yeasts: (magic, Fleischman's)
      (b) to bread making
- F. Main uses — (see wines. beer, etc., under economic phases of bacteriology)
  - (A) Alcohol
    - a. in wines, beers, milk drinks
    - b. held in solutions; escapes from bread during baking
    - c. proportion and rate varies — with type of yeast
      — with substratum used
  - (B) CO_2
    - a. sparkle of wines
    - b. pop, if liquid bottled before fermentation ceases
    - c. texture of bread; effect of baking process
    - d. formerly used as test for sugar in urine in diabetes; uncertain?
- G. Types of yeast (see beers, wines for related types; also for "pseudo yeasts" and "torulas")
  - (A) Bread yeasts
    - a. common forms
      - (a) leaven
      - (b) barm or hop yeast
      - (c) compressed yeast
      - (d) dry yeast
    - b. wild yeasts
      - (a) salt-rising
      - (b) grape juice
      - (c) "witch yeast"
    - c. relative purity of various commercial forms
      - (a) effect of accompanying bacteria

- d. additions to yeasts
    - (a) advantages of
        - 1. hops
        - 2. malt
        - 3. potato
- e. yeast substitutes
    - (a) chemicals
    - (b) aeration, as aerated bread

(B) Involution forms — causes

(C) Keeping qualities of various types
- a. under ordinary or practical conditions
- b. survival periods on record

H. Pathogenic and injurious yeasts
- (A) Relatively few authentic pathological cases
- (B) Yeasts may cause ulcers, "thrush," tumor, and skin affections (blastomycosis)
- (C) Mosquito bite; initial swellings possibly due to yeasts inserted
- (D) Symbiotic? relation in sapsucking insects
    - a. partially digest foods
    - b. keep down bacteria

I. Yeasts as valuable introduction to study of bacteria
- (A) Safe to handle experimentally (testing gas, etc.)
- (B) More favorable for observing growth changes
    - a larger size
    - b. growth rapid
    - c. cells non-motile
- (C) Important cell characters readily demonstrable
    - a. cell wall
    - b. cytoplasmic membrane (plasmolyzed)
    - c. nucleus (easily seen in cheese, or milk yeasts, with methylene blue)
    - d. vacuole
    - e. involution forms
- (D) Illustrate importance of careful technique of fixing and staining (shape of cells and plasmolysis)
- (E) Important physiological principles can be demonstrated in one laboratory period
    - a. difference in food values of various substances or media
    - b. accumulation and determination of wastes and by-products
    - c. diagnostic value of differential results (% CO_2 in gas eliminated)
    - d. effect of temperature on growth and cell activity; on viability

LABORATORY EXERCISES WITH YEASTS

1. With the platinum loop place, as shown, a little yeast growing in molasses and water (3 teaspoonfuls of molasses to a glass, 24°-40°) on a slide. What cell parts are visible? Add a little iodine. Locate one or two of the accompanying starch grains (by their iodine reaction). Draw 2-3 yeast cells with one starch grain, to give an idea of the relative size of these small cells; label each cell part observed.

2. Focus carefully on the central body in these iodine-stained cells. By slight focal changes (fine-adjustment screw) determine whether it is a nucleus or a vacuole. Compare with the slide specially stained for nuclei set up for observation.

3. Stain yeast mixed with starch or flour (not sugar)* with methylene blue, as shown, following the printed directions for staining bacteria (p. 33). What changes are to be observed in cell outlines? In visible cell parts? Find for these a common explanation.

*Sugary smears do not always stick to the slide — especially for beginners.

4. With a sterile toothpick rub a little cream cheese on a clean glass slide. Wash in alcohol, as shown, 1-2 minutes, to fix the organisms to the slide. Drain dry. Wash in xylol, as shown, ½-1 minute to remove most of the fat. Drain dry. Stain with methylene blue.

Examine under the oil-immersion lens (see p. 8). How do these differ from the compressed yeast cells already examined (2)?

5. Plan experiments for the following:

(1) Is CO_2 formed by yeasts?
(2) How much of the gas given off by yeasts is CO_2?
(3) What is the relative availability of the following foods: dextrose (or glucose), molasses, cane sugar, starch, and flour?
(4) Show that temperatures affect (a) the growth of yeasts; (b) the viability of yeasts.

What materials will you need to set up your experiment? Make out a complete list before you leave, that the materials may be in readiness for you at the beginning of the next period.

Important questions to be settled before the experiments are planned are:

How can we detect CO_2 (for exper. 1)?
How can we estimate or compare the rate of yeast action (for exper. 3 and 4)?
Sodium hydroxide (2 to 5% solution) absorbs CO_2; how can we use this in exper. 2?

6. Set up promptly the experiment assigned to you during the last lesson. The tubes, yeast, etc., you asked for are on the tables. As soon as the first change occurs, record the exact time and notify the other members of the class at once. Report any significant later changes also. At the close of the period be ready to report upon the experiment in full.

7. Write up one experiment for each of the four problems (Each one on a separate paper, as they will be corrected and returned separately). Select but one, even though the problem was answered in more than one way. Have them ready to hand in one week from to-day.

YEAST DEMONSTRATION

1. Yeasts — with potato starch. If not previously completed, draw one starch grain with several yeast cells (so as to show the relative size of yeasts).

2. Yeast cells from milk (or cheese) stained with methylene blue. Note how definitely the nucleus has taken the stain. Add (unless previously drawn) a drawing of one of these cells to your yeast drawings, labelling the nucleus. (The background is also blue, owing [partly, at least] to the affinity of the milk proteins for this stain.)

3. Yeast — commercial forms: compressed yeast; magic yeast; beer yeast, just obtained from a near-by brewery.

4. A slide showing a wide range in the shape and the size of the yeast cells — involution forms. Such variations are common in old cultures, with accumulated wastes, etc. Similar changes in bacteria (e.g., diphtheria) may make the diagnosis of disease difficult.

5. Old molasses mixture, with a scum of yeasts, bacteria, etc., on the surface. This heavy film is called a zooglea. If you have time, examine this for involution forms of yeast.

6. Beer yeast. Note how large and round these cells are. Are bacteria present? How do you account for this?

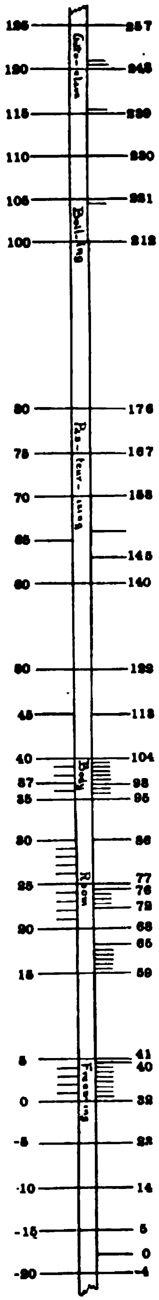
7. Bread yeast. How many types of yeast are here? How does bread compare with beer yeast in the number of bacteria? starch grains?

8. Magic yeast. How does this yeast differ from the two just examined?

9. A red yeast; the most common pigments in yeasts are red and pink; they are quite easily isolated from the air by exposing agar plates. Note that the cells seem smaller than the cells of commercial yeasts, and less granular in character.

VI. MEASURES, MEDIA, STAINS, ETC.

Temperature. The accompanying thermometer slips give equivalents (Centigrade and Fahrenheit) for the temperatures most often used in bacteriological work (ranging from —20° C. to 125° C.). The middle column indicates the main temperature groups: body, pasteurizing, freezing, etc. On the reverse side are rules for deriving other equivalent temperatures.



To convert degrees Fahrenheit into degrees Centigrade:

subtract 32,
multiply by 5,
divide by 9

To convert degrees Centigrade into degrees Fahrenheit:

multiply by 9,
divide by 5,
add 32

Length. The inch and its most-used divisions (1/8, 1/12) are too long for such tiny micro-organisms as bacteria; it is, of course, necessary to have a unit small enough to be *repeated* in the length of the organism to be measured. The centimeter (1/100 of a meter) is subdivided into 10 divisions: millimeters. Each of these is again subdivided into 1000 parts; each of these final divisions is called a μ or a micro-millimeter; a μ is therefore 1/10,000 of a centimeter.

1 in. = 2.54 cm.
or
1 cm. = about 1/2.5 of one inch
1 mm. = 1/10 of one cm. = about 1/25 of one inch
1 μ = 1/000 of 1 mm. = about 1/25,000 of one inch.

Weight. The unit of weight is the metric system unit, the gram. 1000 grams = 1 kilo (kilogram).

Keep in mind that about 30 (28.8) grams make an ounce. How does a kilo compare with a pound? How many ounces in a kilo?

The gram itself is sometimes divided. The milligram (1/1000 of a gram) is the common unit for amounts lighter than a gram.

Volume. 1000 cc. = 1 liter.

1 cubic centimeter of water at 4° C. weighs 1 gram. How many cubic centimeters in a kilo? How many grams in 1 liter of water? How would the number of grams in a liter of broth, agar, or carbolic solution, compare with this?

To visualize these volume measures, remember that a cubic centimeter is about 16 drops; a liter is a little more than a quart (a quart is about 960 c.c.).

Per cent solutions. In making up solutions it is the common laboratory practice to use grams and cubic centimeters interchangeably. For example, in making up a 5% sugar solution we would weigh out 5 grams of sugar and add that to 95 c.c of water. In handling such liquid disinfectants as carbolic acid, the custom is to take 5 c.c by *bulk* of the heavy acid liquid, adding 95 c.c. of water. Here we have, of course, more than 5%; the difference, however, in disinfectants, is on the safe side, making the solution stronger, not weaker.

Securing solutions of designated proportions. 1. It is often necessary to change a solution of known strength to another strength or per cent. Occasionally disinfectants, etc., will bear such labels as, "To one quart of water add one-fourth of a bottle," etc., or "To any bulk of disinfectant add four times that amount of water." There will be many occasions, however, (e.g., making stains) when you will need to work more exactly — in percentages. The "rule of three" may always be relied upon — not only to give you your per cent, but in the desired bulk. For example, if you need 500 c.c. of a 5% solution of formalin and your stock formalin is a 40% solution, your mathematical arrangement would read: 40% : 5% :: 500 c.c.: (?) or 62.5 c.c. You would then take 62.5 c.c. of the 40% solution, add water to bring it up to 500 c.c., thus securing 500 c.c. of a 5% solution.

2. (Optional.) Solutions based on 95% alcohol are often puzzling. Phenolphthalein, for instance, has to be dissolved in 95% alcohol (it will not dissolve in 30% or 50% alcohol); it is then usually diluted to give 1/2 of 1% solution in 30 or 50% alcohol. If you wish 200 c.c. of a half per cent phenolphthalein solution, how much phenolphthalein will you need? how much 95% alcohol?

3. To get solutions of the required strength it is usually much quicker to follow a very old but little-used laboratory rule: to dilute to any strength take a number of units (oz., c.c., etc.) to correspond to the dilution desired and add water (or designated solvent) to bring the bulk up to the number of units indicated by the original substance. To illustrate: to get 12% alcohol from 95%, pour 12 c.c. of alcohol into a graduate, add enough water to fill your graduate up to 95 c.c. You will then have 95 c.c. of 12% alcohol.

How will you make 2½% formalin from 40%?

This method is not so well adapted for expensive materials; why not?

COMMON MATERIALS USED IN THE STUDY OF MICRO-ORGANISMS

1. **Gelatine.** An albuminoid, used (1) to give a solid medium for the study of bacterial colony characters and (2) to demonstrate for a given bacterium the presence of liquefying enzymes.

This form of gelatine (Gold seal) is used in bacteriology, 10 to 15% usually. It is added to broth and used as shown here in plates or Petri dishes or in tubes with straight or slanted surfaces.

2. **Agar or agar-agar.** A glutinous substance expressed from red seaweed, which is sold in shreds or stringy masses or as a powder. It is used as gelatine is used; 1 to 1½% is sufficient, however, to give a firm medium.

3. Seaweed from which agar is made. Some forms of this are eaten (dulce in Ireland; many forms in China, Hawaii, etc.).

4. Strip agar, an exudate of agar, obtained by pressure, soaking in *fresh* water, etc. Dip a thread of agar into water. Notice how rapidly it absorbs water. (It is now advised widely for constipation, as it is a comparatively indigestible carbohydrate, but gives bulk and holds water, thus acting as a mechanical irritant as well as having a favorable effect with regard to the watery content of the intestine.)

5. Agar is with difficulty used as food by bacteria, and it is not liquefied by bacterial enzyme action as gelatine is. (Do not confuse the liquefaction of gelatine with the condensation of water in an agar plate or the expressed water at the base of the slanted surface of an agar tube.) Agar solidifies at about 38° C. and remains solid until nearly boiling temperature is reached, while gelatine liquefies at slightly above room temperature. Which medium is preferable for studying pathogenic organisms? § for making plate counts? § Which medium has the greater differential value? §

6. **Litmus,** a plant extract, sensitive to acids and alkalies. Most students are already familiar with litmus paper — paper colored with litmus solutions. These are papers soaked in acid litmus solution (red), alkaline litmus solution (blue), or neutral litmus solution (pink-purple). If not familiar with the action of litmus paper, add a drop of lactic acid to litmus paper — each color. For alkali effects use ammonia. For what would neutral litmus paper be useful? Why is litmus called an indicator?

For bacteriological work, blue litmus is usually made up into a stock (water) solution — 5 per cent. This is used to color agar and (sterile) milk. A litmus medium usually contains a sugar also. It is usually used to show that the bacteria can (or cannot) utilize designated sugars or carbohydrates, such as lactose, dextrose, and saccharose; evidence of such power is given when the litmus medium turns pink or reddish, due to lactic (or other) acids formed from the lactose or other test substance.

7. **Phenolphthalein.** There are indicators more sensitive than litmus. Phenolphthalein, purchased in powder form, is made up into alcoholic solution — usually ½% in 50% alcohol. In a series of weak solutions, phenolphthalein will continue to differentiate between the acids and alkalies long after litmus ceases to react. (A medium that is neutral to litmus is usually .8 to 1.2 acid to phenolphthalein.) In acid or neutral solutions, phenolphthalein remains colorless; in alkaline solutions, it turns pink, the depth of color ranges from faint pink to raspberry or even a purplish red, depending upon the strength of the alkali. See accompanying dishes of acids, alkalies, and (neutral) water treated with phenolphthalein.

Phenolphthalein has long been used to secure bacterial media of quite definite acid or alkaline condition. Standard acid and alkaline solutions are made for testing the condition of the medium. These solutions are usually sodium hydroxide, and either hydrochloric acid or oxalic acid. Such solutions often range from a fraction of 1 per cent to about 4 per cent. (These solutions are made up on the basis of the atomic weights of the substances, but modified to correspond with the hydrogen atoms represented.) For these solutions a graded burette is used. An arbitrary scale has been adopted for measuring the acidity or alkalinity of a medium. For example: a medium that is 1.5 acid is one that has so much acid in

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The mark § indicates that if the answer is not obvious now, it may be found during the next few lessons.

it that it takes 1.5 c.c. of the standard alkaline solution to neutralize the acid in 100 c.c. of it.* (For economy a smaller amount (5 c.c.) is used in actual work; the standard solutions are diluted too, making the test substance more delicate as an indicator.) Alkaline media are less often used in bacteriological work; they are measured against standard solutions of acid. To secure media of desired acid or alkaline reactions, it is often necessary to add one of our standard solutions to the medium. How much is determined by comparing the reaction of the medium with the desired reaction. (See Jordan for details.) Or, again, if we wish to find how much acid or alkali a given culture of bacteria has formed, we measure out a given amount (usually 5 c.c.) of the culture medium, dilute it in water (usually 45 c.c.) and add phenolphthalein (3 drops). If the medium turns pink, we know it is alkaline. How alkaline can be determined by seeing how much of our standard solution of acid is necessary to make it lose its pink color. If it does not turn pink, we know it is acid. How acid can be found by running enough of the standard alkali solution to bring it to an alkaline condition with the attending pink reaction.

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*This is not per cent. For example, the standard solution is about 4 per cent solution; 1 c.c. of this per 100 c.c. is consequently very far from 1 per cent. A reaction, therefore, of 1.5 cannot be read 1.5 per cent acid, as is the custom of some workers. It should be read simply 1 point 5 acid.

MAKING BROTH AND AGAR

1. Plan your work so as to bring the work within the shortest compass. Read all the directions through first. Melt the agar while the other work is progressing. During unoccupied intervals, (e.g., while waiting for the broth to filter), make the plugs (as shown) for your tubes, prepare the hot-absorbent cotton filter for agar, etc.

2. If you are working in groups, make sure that you do a little of every part of the work. Otherwise you gain little more by this exercise than if the media were all made for you entirely outside of class.

3. Read the directions for making broth and agar in the usual way.

4. Because of the present almost prohibitive "war prices" of peptone we are substituting for the usual ingredients a clear commercial soup plus sugar (dextrose). (This is a very simple process, and suitable for high school classes where the occasional demands make it not worth while to buy a whole can of extract or a whole bottle of peptone. The modified directions follow.

5. To one half pint can of clear soup add enough water to bring the bulk up to 1 liter (about 1 part soup, 4 parts water). To this add ½% dextrose. Heat until the dextrose is dissolved, filter through filter paper. If not perfectly clear, repeat the filtering process. Do not cook too long, or you will lose too much water. (What two possible disadvantages in that?) Test with litmus paper to make sure that you have the generally favorable reaction for bacteria: neutral, or faintly alkaline. Save out 500 c.c. to use in making agar. Tube the rest (about 1 in. to a tube), plug, and sterilize 15 minutes in the autoclave.

6. Agar is made by adding 1½% agar to broth. How many grams of agar must we add to the 500 c.c. of broth reserved for making agar?

It takes some time to liquefy agar. Boiling over an open flame is tedious, and attended by danger of burning. We find it simpler to heat the required amount of agar in a small amount of water (15 gm. agar to 200 c.c. of water) in a cotton-plugged bottle, and have it dissolved ready for use. When ready to make agar, appropriate quantities of this agar can be again melted in the Arnold or autoclave while the broth is being prepared; then to the boiling hot broth add the melted agar, filtered while hot through a hot, wet filter of absorbent cotton kept in the Arnold until ready for use. Agar has little if any effect upon acidity; it is not customary

to test again with litmus. This method means that you used more than 500 c.c. of water for your 7½ grams of agar. So much water is lost during this process, that you will find you have lost the extra water and more by the time the agar is ready to tube. For agar plates, the tubes should be slightly more than half full; for slants the tubes need be but one-third full. Make about 5 of latter, and tube the rest to use for plates. Sterilize, etc., as you did the broth.

PIPETTES

1. Pipettes used in making dilutions of different strengths for milk and water plates. Three different kinds of 1 c.c. pipettes are in this rack. In the pipette marked A the 1 c.c. space extends from the pointed tip to the upper level indicated by a faint scratch around the tube and the mark 1 c.c. (These are made to deliver 1 c.c.; it is not necessary to blow through the pipette.) In B, this space is still further subdivided into tenths of a c.c.; in C, the ultimate divisions are hundredths of a c.c.

2. Practice drawing 1 c.c. of water up into a pipette; let it out by tenths, stopping completely at each tenth mark. Repeat, until you are sure you can control the amount leaving the pipette, so that next lesson the sterilized pipettes will not be wasted by errors.

Caution. Use the first finger, *not* the thumb, to cover the pipette. Using the thumb tightens the hand too much for the delicate manipulation often necessary in bacteriological work. Hold the pipette near the top, so that the first finger rests comfortably on the top of the pipette. In measuring the outgo, lift the finger, the knuckle part, not the nail end first, as the skin near the nail is harder and gives a less delicate control than the part near the middle of the joint.

3. A new type of pipette which protects the user. The two bulbs take care of excess liquid (cultures, etc.), and do not allow it to enter the mouth. A cotton plug is sometimes used at the mouth end of the other pipettes. That is less satisfactory, and it is often quite troublesome to insert the cotton and to clean it out after using.

STAINS

1. Bacterial stains, such as fuchsin and methylene blue, are purchased usually in a powdered condition. These are then made up into stock alcoholic solutions — saturated solutions in 95% alcohol usually. How can we be sure we have saturated solutions? (Hold to the light, and tilt the bottles slightly.)

2. These saturated solutions are then made up into weaker watery solutions ready for use.

3. Certain chemicals increase the staining qualities of bacteria. Such substances are called mordants. Carbolic acid is the mordant commonly used with fuchsin; potassium hydroxide is used with methylene blue. With very resistant bacteria (such as the waxy-coated tuberculosis bacilli) heat is also applied, acting as a mordant. (See directions for staining tuberculosis.)

STAINING EXERCISES

1. **Methylene Blue.**

(1) On a clean slide place a loop of broth containing bacteria. If the bacteria are in a solid medium, place a drop of sterile water (or broth) on the slide, and with a sterile needle transfer a minute portion of the culture to the water, spreading it throughout the drop.

(2) Allow the film to dry at room temperature.

(3) Fix the organisms to the glass by passing it three times through a flame. (Too great heat will shrivel them. Why?)

(4) Add enough stain (methylene blue) to cover the film well, and let it stand 1 to 3 minutes.

(5) Wash in water; with clean slides the film will show the stain, and the rest of the glass will be perfectly clear.

(6) Dry, by draining, by pressing gently, film down, on a piece of filter paper.

(7) Add a drop of water, cover with a clean cover-glass, and examine with the high power.

2. **Fuchsin.** For one of your slides fixed by heat, use carbol-fuchsin. As shown, flood with sterile water (to prevent a precipitate from settling on the smear), stain ½ to 1 minute, wash in water, and drain or blot dry, as usual.

3. **India ink stain.** (Optional.) (1 part India ink to 4 parts of water, sterilized in the autoclave.)

Place a loop of India ink on a clean slide. To this add a loop of the bacterial culture, mixing it, as shown, so that there is a range of thick and thin places on the slide, some of which will be dark enough to make a favorable background, yet thin enough to allow enough light to pass through to illumine the organisms. When dry, add oil and examine. Do not add water; that dissolves the ink immediately. What are the advantages of this stain? Any disadvantages?

4. **Corrosive sublimate as a fixing agent.** Heat is not always a satisfactory agent for fixing micro-organisms to the slide, e.g., milk, for which we employ alcohol. Some stains, e.g., Jenner's, are used in strong alcoholic solutions, and do not require any preparatory "fixing." Most of these require care to prevent the deposition of sediment on the slide (filtering the stain, covering to prevent evaporation of the alcohol during staining, etc.).

A "fixing" agent in quite common use is corrosive sublimate, saturate water solution. Fix with this, as shown, wash *thoroughly,* changing the water at least once (to avoid crystals and precipitation), dry at room temperature, and stain as usual with methylene blue. Compare this with a control heat-fixed slide. What differences?

VII. BACTERIA (AND PROTOZOA)

(General preliminary discussion)

Illustrative material: Charts showing (1) chemical analysis of bacteria, e.g., tuberculosis; (2) morphological types.

A. Relative position of bacteria and protozoa in biological classifications
    (A) With relation to other plant and animal types
    (B) With relation to each other
B. General comparison of bacteria and protozoa
    (A) Terms common to each (germ, microbe, micro-organism)
    (B) Size
    (C) Cell parts — wall
        — vacuole
        — nucleus
    (D) Effect of drugs and stains *(in vivo)*
    (E) Common types of each
        a. bacteria (including higher bacteria)
        b. protozoa (important pathogenic types)
C. Distribution of bacteria
    (A) Universal presence

- (B) Distinguish between habitat and temporary or accidental presence

D. Classification of bacteria
- (A) Main genera with approximate number of each
- (B) Generic differences
  - a. mainly morphological
    - (a) shape of individual cells
    - (b) relative arrangement of cells
    - (c) number and position of cilia or flagella
  - b. not entirely satisfactory
    - (a) shape and size not distinctive
    - (b) mutation and involution forms
    - (c) flagella
      - 1. inconstant
      - 2. not easy to demonstrate
- (C) Specific differences
  - a. mainly physiological
    - (a) relatively fixed — serve to differentiate pseudo-forms, which are alike morphologically rather than pathologically
  - b. morphological characters (capsule, square ends, etc.) also used

E. Size of bacteria
- (A) Contrast molds; yeasts
- (B) Units of measure used
  - a. micron or micro-millimeter
- (C) Range in size
  - a. longest known
  - b. smallest yet isolated
  - c. ultra-microscopic organisms
  - d. compare size and weight

F. Cell parts (terminology of various standard authors for cell wall, ectoplasm, capsule, etc., not interchangeable)
- (A) Cell wall
- (B) Additional capsule
  - a. relation to food, to age
  - b. staining qualities
- (C) Flagella
  - a. really protoplasmic projections
  - b. rate of movement
- (D) Cytoplasmic membrane
- (E) Cytoplasm
  - a. included granules
    - (a) metachromatic: nuclear
      - 1. staining qualities
      - 2. concerned with cell division
      - 3. some refractile when unstained
    - (b) food granules (starch, fat, glycogen); vary in staining properties
      - 1. from each other
      - 2. from nuclear granules
- (F) Nucleus — represented by scattered chromatin
  — sometimes visible just before spore formation
  — basic or nuclear stains used as bacterial stains
- (G) Vacuoles
- (H) Pigment — not in plastids
  — often outside the cells (excretions)

G. Cell division

- (A) Probably related to metachromatic granules; compare mitosis in higher plants
- (B) Time required — estimate possible increase in 6 hrs., 1 day
- (C) Rate — affected by
    - a. food
    - b. water supply
    - c. accumulated wastes
    - d. temperature

H. Spore production
- (A) Bacilli only
- (B) Number per cell
- (C) Reproduction not multiplication except in "higher bacteria"
- (D) Characteristic appearance
    - a. position of spores in various types
    - b. refractile (when unstained)
    - c. staining qualities
- (E) Few pathogenic bacteria produce spores
- (F) Relate
    - a. to disinfection
    - b. to pasteurization

I. Chemical composition
- (A) Mainly H_2O
- (B) Other organic substances demonstrated in:
    - a. wall
        - (a) mainly protein
        - (b) also hemi-cellulose, wax, etc.
    - b. cytoplasm often containing
        - (a) various albuminous substances
        - (b) fats
        - (c) glycogen
        - (d) nuclein and nuclein bases
    - c. inorganic substances
        - (a) minerals, 1-8 % of dry cell substance
        - (b) special minerals — prominent different species (sulphur bacteria, etc.)
        - (c) minerals commonly present
            - 1. salts of potassium, phosphorus, chlorine, calcium, sulphur, and silica
            - 2. phosphoric acid; often ½ of dry weight

GENERAL DEMONSTRATION OF BACTERIA

(Appearance (including colony characters) on agar, and general cell morphology.)

1. Anthrax, growing on an agar slant. Can you see why whitish patches may develop in the throat in diphtheria? (Such patches are bacteria plus mucus and dead cells.)

2. This microscope shows anthrax bacteria growing in long chains (×1200, oil-immersion lens), stained. Imagine one of the single rods divided into 1200 parts, and you will have the approximate size of this organism.

3. Agar slant of a pigment-forming bacterium, a coccus organism.

4. The same coccus organisms (×1200), lying in characteristic groupings 2, 4, and 8; stained red.

5. Slide showing huge spiral organisms obtained from marshy pool. Note the several darker spots in these large cells; they are probably nuclear.

6. Small spirals of but one curve or turn each — more comma-shaped than spiral; these are cholera organisms.

7. A bacterium isolated from water, containing quite definite nuclei in the center; in most bacteria the nuclear material (and therefore the stain, also) is quite evenly scattered through the cell.

8. Bacilli beginning to divide, each cell or bacillus forming two organisms only. Find some that show slight indentations where separation will finally occur.

9. Bacilli showing vacuoles. See accompanying illustration.

10. The previous slides have all shown stained bacteria. The following show bacteria mixed with India ink. The background is therefore dark, while the colorless or whitish bacteria may be clearly seen against the India ink background. This first microscope shows bacilli.

11. This microscope shows coccus organisms in somewhat twisted chains (Streptococcus), also unstained, on an India ink background.

12. Streptococcus stained with a special stain to bring out the hazy capsule surrounding each organism. It appears as a long hazy band each side of the chains shown here. See illustration also.

13. Flagella on long rods or bacilli isolated from maple sugar. Compare with illustration.

14. Look at the various organisms pictured in this book, to see other methods of flagella arrangement. Some authors use these differences as a basis for classification. See accompanying chart.

15. Bacilli showing spores in the center of the cell — and but one to a cell. This is the most common method of spore production among bacteria.

16. Tetanus or lock-jaw organisms, which form spores at one end of the narrow rod. How does spore formation affect the shape of these cells?

17. A classification of bacteria based primarily on (1) shape, (2) relative arrangement of the individual cells.

18. These characters are insufficient for distinguishing the hundreds of bacterial species. One of the most important aids is the colony character when grown on agar. The accompanying card or chart (Society of American Bacteriologists) gives the common descriptive terms applied to such growths. Those checked are the ones most commonly used.

On this table are about 30 agar plates containing bacteria. (The air plates, p. 54, are usually made out of sequence and collected in time for this exercise.) Various types have been ringed with a wax pencil to aid you in selecting those illustrating the terms described on the chart. Use a hand lens for all small colonies. Note that a single colony may often best be described by two or more terms; e.g., Plate 1 shows spreading, effuse colonies with fimbriate (fringed) margins. List the terms fitting the numbered plates; later in the period we will compare the terms selected by the members of the class.

If today's collection does not include examples of the following, keep on the lookout for them, and hand them in for class observation as you find them.

pigmented
myceloid
mixed
wrinkled
concentric
thick-margined
umbonate
moist
cretaceous
opaque
transparent
radiate
solid
fimbriate
spreading
effuse
granular
warty
small
minute
punctuate

VIII. VITAL PHENOMENA EXHIBITED BY BACTERIA

Illustrative material:
- Bacteria — slants, showing pigment.
- Chart — giving formulas and equations for various processes; (1) cleavage and oxidation of food substances; (2) different ways of decomposing a given substance (dextrose).

A. Several characteristic bacterial manifestations
- (A) Motility
    - a. due to flagella or cilia
        - (a) characteristic types of movement
        - (b) compare flexuous bodies of protozoa
    - b. confused with Brownian movement
    - c. effect of microscope on apparent rate
- (B) Light
    - a. sufficient
        - (a) to cause luminous appearance
        - (b) to take own photograph
    - b. characteristic of *living* substance
        - (a) not in filtrate
        - (b) salts necessary
            - 1. ocean, marshes, etc.
            - 2. salted fish or meat
        - (c) not a constant character
- (C) Heat
    - a. compare heat evolved by all living cells
    - b. shown in cultures by delicate thermometers
    - c. exhibited on large scale
        - (a) manure piles
        - (b) silo
        - (c) preparation of tobacco, cocoa, etc.
- (D) Pigment
    - a. range of colors
    - b. lipochromes (chiefly) — common in plants (carrot) and animals (egg yolk)
    - c. distinguished by solvents — water, alcohol, etc.
    - d. no known importance (except bacterio-purpurin, which functions as chlorophyll does?)
    - e. color affected by oxygen — oxidation of excreted wastes?
    - f. presence erratic; affected by
        - (a) temperature
        - (b) oxygen
        - (c) food
        - (d) antiseptics, etc.
- (E) Aromatic substances, etc.
    - a. odor of wines and beers (in part)
    - b. butter: butyric acid; fishy flavor
    - c. indol, skatol, etc.
    - d. freshly-plowed earth or smell of spring
- (F) Toxins and ptomaines (often confused)
    - a. likenesses
        - (a) related to protein-decomposition
        - (b) may be injurious to man
        - (c) may accumulate in milk, meat, etc.
    - b. toxins
        - (a) very poisonous; compare tetanus toxin with known drug poisons
        - (b) pure toxins yet secured?
        - (c) result of cell metabolism
    - c. ptomaines

- (a) chiefly formed *outside of cells*
    - 1. large protein molecule too large for absorption
    - 2. most prominent in protein foods favorable for bacterial growth (milk, milk products, oysters, meat)
- (b) many definitely known (pure form)
    - 1. alkaloid in character
    - 2. chemical formulas known
    - 3. most are harmless, or rendered so by oxidation
- (c) compare leucomaines of human alimentary tract
- (d) too loosely applied to digestive difficulties?
- (e) legal phases

B. General discussion of vital phenomena

- (A) Metabolic processes of bacteria resemble those of all other living cells
    - a. breaking down of complex food substances for use in cell
        - (a) accomplished by simple cleavage, oxidation, etc.
        - (b) attended by liberation of heat and energy
    - b. assimilation of materials into the cell substance (nucleoplasm, etc.)
    - c. synthesis of complex food and storage material (glycogen)
- (B) Activities and changes generally discussed under bacterial action
    - a. enzymes necessary; these and other substances useful in cell economy generally termed secretions — not necessarily retained in the cell; e.g., lactase
    - b. excretions and other wastes (e.g., mineral crystals); term generally applied to substances eliminated from cells and of no further use in cell economy; toxins, CO_2 (?), excess water
    - c. disintegration products
        - (a) types
            - 1. of substances never part of, or even inside the cell — mainly preparatory to absorption as foods
            - 2. of substances forming the cell, or stored in them — valuable for providing heat, energy, and food
        - (b) accomplished mainly by enzymes
            - 1. cleavage, oxidation, etc.
            - 2. energy obtained varies greatly; compare yeast (cleavage of sugar) with certain molds (oxidation)
        - (c) major process — popularly termed fermentation (See yeasts for limitations often implied in this term.) In these lectures fermentation will be used in the broadest sense: for the decomposition of proteins as well as carbohydrates, usually attended by the formation of gas, or acid, or both.* Putrefaction is quite generally applied to those malodorous changes accompanying the decomposition of proteins, under more or less anaerobic conditions
            - 1. varied enzymes connected with fermentative changes
            - 2. effect of oxygen upon resulting gases
            - 3. types of fermentation often named from end or prominent product: alcoholic, acetic, lactic, ammoniacal

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*Many who insist upon gas as a concomitant of fermentation accept without question the term fermentation for the vinegar process, though the products are acid and water — without any gas. As the term is used in this text, the fermentative result may be alkaline and not acid at all, as in "ammoniacal fermentation."

C. Interpretation of vital phenomena
  (A) Vary with organisms
    a. species itself
    b. adaptability and constancy of organism
  (B) Influenced by environment; illustrate for
    a. oxygen
    b. temperature
    c. media
      (a) kind
      (b) concentration
    d. bacterial products
  (C) Diagnostic value
    a. *complex* of fermentative and other phenomena important
    b. incomplete without morphological characters
    c. related to other recognized tests, e.g., pathogenicity
    d. important
      (a) naming organisms
      (b) diagnosing disease
      (c) sanitary tests

VARIOUS VITAL PHENOMENA INCLUDING RESULTS IN SOME COMMON MEDIA

Motility. a. Carmine, a pigment composed of very fine particles.

b. Carmine particles in a drop of water. Note under the microscope the dancing motion of each particle — motion without progression.* This phenomenon is often noticed in suspensions of small particles, and is named (for an early investigator) Brownian movement.

This movement without any change in relative position of these small bodies is attributed to (1) the molecular motion characteristic of molecules in general, and so exhibited more or less by any tiny particles; or (2) to differences in surface tension, a torsion or twisting resulting from the unequal wetting of the irregular surfaces of the particles.

c. Mount, as shown, a hanging drop of old yeast or stagnant water, seal with vaseline, and study (high power) until you are sure it does not sway too much (too large a drop) nor shift (imperfectly sealed). Find (1) organisms that show only Brownian movement and (2) other organisms that are truly motile, and change their relative positions.

Pigment Formation. a. Slants of several pure cultures, showing one non-pigmented and several pigmented types: e.g., red, *B. prodigiosus;* yellow, *Sarcina lutea;* and green, *B. pyocyaneus.*

b. Solubility of pigment in water. Compare *B. pyocyaneus* and *B. prodigiosus* with regard to the position of the organisms on the slant and the distribution of color in the tube. In which is the pigment soluble in water?

c. Other pigments are soluble in alcohol, ether, etc. To one slant of *B. prodigiosus* has been added some alcohol. Is that pigment soluble in alcohol?

Broth Characters. Pick out from these tubes, one to three days old, those that illustrate the following terms: clear, (with or without sediment) slight turbidity, heavy turbidity, pellicle, slight sediment, heavy sediment, viscid sediment, granular sediment, and flocculent sediment.

Acid and Alkali Production. a. Does the litmus paper indicate any difference in the amount of acid produced in plain broth by these two cultures? Any for the same organism growing in plain broth and in sugar broth? For the same organism in plain bouillon and in milk? Measured quantitatively with phenolphthalein, a

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*Pressure upon the cover-glass may set up currents, of course; wait until they cease. Currents due to the evaporation from the side of the cover-glass may also occur. There is no individual or apparently self-directed movement of the organisms or particles in Brownian movement.

decided difference may be shown; e.g., sugar media often contains four to seven times the acid formed in ordinary plain bouillon by the same bacterium.

b. Acid formation in milk may be accompanied by changes in consistency; select tubes which show slight curdling, firm coagulum, and by expression of the whey from a hard, firm mass at the bottom or the side of the tube, or a curious cobwebby substance. When ammonia and other alkalies result from the decomposition of the media, the litmus milk becomes a deeper blue as shown by this tube of *B. subtilis*. Note the litmus milk tubes where the milk is now white (or the litmus agar tubes which are now almost the color of plain agar). How do you interpret this?

Growth on potato: sterile slant before inoculation
slants showing bacteria — pigmented
— non-pigmented
slants partly disintegrated, showing digestion of the potato starch. Many bacteria fail to digest potato.

Gelatine: Compare the tubes containing stab cultures of different bacteria with the illustrations in the text-book. Do all bacteria liquefy gelatine in the same way? Describe one of those shown.

Oxygen relations: (a) to growth. A streak of *B. prodigiosus* was made across this plate of agar with a platinum needle. A cover-glass was then pressed firmly against the agar covering part of the streak. The plate was then incubated at room temperature for two days. Compare growth and pigment formation in the parts possessing and lacking oxygen.

(b) to spore formation. Explain the heavier spore production in the outer edge of these dishes (Petri dishes, watch-glasses) of mold.

Haemolysis: Besides the enzymes which disintegrate common carbohydrates (starch, sugar) and proteins (gelatine, casein), there are many other bacterial enzymes. The bacteria growing on this plate of blood-agar have dissolved and destroyed the red blood cells near them, leaving a clear or haemolyzed area around each colony. What harm might such bacteria do in the animal body? Why do some authors consider haemolysis an indication of pathogenic properties? §

NAMING UNKNOWN ORGANISMS

1. Some of the descriptive terms for bacteria were given in an earlier demonstration. (See also Jordan, Chap. II, or any preferred text.) The reverse of this descriptive chart shown at that time indicates other characters of value in nomenclature and diagnosis.

2. Unknown organism (×1200), note the shape of the individual cells. Does it produce spores? Are the cells single or in twos, or packets (4, 8), or irregular masses, or in definite chains (more than 4). Is it gram-positive? Describe the flagella.

3. Following is a series of culture media showing the effects produced by this same unknown organism.
How does it grow in plain bouillon? In sugar media?
Any pigment on agar?
Does it liquefy gelatine?
How does it affect litmus milk? plain milk?
Is the growth better on the room temperature slants or on the 37° slants?

4. Using these facts, at what name do you finally arrive in this classification of bacteria (Journal of Bacteriology, September, 1917).

5. Look up the genus selected in Chester's Manual of Determinative Bacteriology; can you determine the species?

IX. EFFECTS OF PHYSICAL AND CHEMICAL AGENTS ON BACTERIA

Illustrative material: Charts giving
- (1) coagulating temperatures of various percentages of albumin
- (2) various pasteurizing temperatures (with time period)

A. Introductory discussion
- (A) Results include
  - a. death of the organisms
  - b. inhibition of their growth
  - c. attenuation of their powers
  - d. changes in their characters (motility, pigment, etc.)
- (B) Agents named to indicate action
  - a. germicide, bactericide, disinfectant; (compare disinfect and sterilize)
  - b. antiseptic (compare aseptic)
  - c. deodorizer
- (C) Effects due to one or more of the following causes:
  - a. interference with normal metabolic processes
    - (a) plasmolysis
    - (b) concentration of cell substances
    - (c) foods oxidized or made insoluble
    - (d) cell substance coagulated, or other unfavorable chemical combinations made
    - (e) oxidation of the cells themselves
    - (f) toxic and irritant action on cell substance

B. Principal agents
- (A) Electricity
  - a. consideration complicated by the accompanying
    - (a) increase in temperature
    - (b) presence of acids
    - (c) electrolytic ozone, chlorine, etc.
  - b. applications; treating — wine (flavors),
    — milk, water, sewage
- (B) Gravity (affects indirectly, e.g., through position)
  - a. specific gravity of bacteria relatively light
    - (a) gas bubbles in pellicle
    - (b) sedimentation
      - 1. slow
      - 2. modified by
        - (1) currents
        - (2) rate of flow
        - (3) motility of bacteria
- (C) Agitation
  - a. separation of bacterial clumps
  - b. wide range in bacterial susceptibility
  - c. destructive effect
    - (a) protracted, violent shaking
    - (b) constant vibration (heavy machinery)
- (D) Pressure
  - a. methods
    - (a) liquids themselves:
      - a. slight effect, as little gas in solution — only gases in solution can affect bacteria
    - (b) compressed gases for application:
      - a. effects due
        - (1) to amount of gas in solution
        - (2) chemical affinities of the gas
  - b. results vary with

            - (a) temperature
            - (b) organisms
            - (c) gas used (See chemical disinfection later)
            - (d) pressure — amount
— duration
        - c. applications: water, milk, canned goods (compare enzymes in substance themselves)
- (E) Light
    - a. sunlight
        - (a) duration
        - (b) intensity
            - 1. seasonal variations
            - 2. affected by intervening matter
                - (1) air — clouds, dust
                - (2) water — turbidity
                  — salts in solution
                - (3) mucus, sputum
        - (c) relative effect on spores and vegetative stage
        - (d) effect of various parts of the spectrum
            - 1. red to green
            - 2. green
            - 3. blue and ultra-violet
        - (e) applications: bedding, ready-made clothing, etc.
    - b. artificial lights
        - (a) ultra-violet light
            - 1. glass and quartz containers
            - 2. rate
            - 3. applications
                - (1) water
                - (2) bacterial preparations (as vaccines)
        - (b) radium and x-rays
            - 1. plant and soil applications (see also soil lecture)
            - 2. treatment of disease
                - (1) relative resistance of tissues and bacteria; dangers attending use
                - (2) present estimate of value
- (F) Removal of H_2O (review importance of H_2O to cell)
    - a. resistance depends on
        - (a) organism, including spore-forming power
        - (b) rate of removal
            - 1. humidity — amount and range
            - 2. heat, light
- (G) Food and similar environmental conditions
    - a. amount of food — compare survival period in water; in distilled water
    - b. concentration of food elements — plasmolysis
    - c. acidity and alkalinity
    - d. lack of essential food elements
        - (a) requirements for
            - 1 energy
            - 2. cell substance
            - 3. stimulants of metabolic activity
    - e. relative sensibility to presence of substances usually classed as foods; e.g., nitrifying bacteria to NH_3
    - f. oxygen an important factor
        - (a) utilization of foods
        - (b) production of pigment
    - g. bacterial by-products

- (a) physiological and morphological changes due to age — as influenced by various media
- (b) incompatibility of certain bacteria — method of obtaining pure cultures

- (H) Temperature
  - a. growth and survival limits
    - (a) maximum, occasionally high (hot springs)
    - (b) minimum, low (existence, if not reproduction)
  - b. high temperatures
    - (a) dry and moist compared
      - 1. relative efficiency
      - 2. penetrating power
      - 3. coagulation point of proteins
      - 4. killing point for bacteria pathogenic to man
    - (b) methods of securing high temperatures
      - 1. without moisture
        - (1) hot air oven — glassware, etc.
        - (2) flame — platinum needles, clothing and other materials to be discarded
      - 2. with moisture
        - (1) pasteurizing
          - — milk (see milk lecture)
            - — death point of disease organisms often transferred by milk
            - — effect of "film" on temperature record
        - (2) boiling — water (see lecture on water)
        - (3) streaming steam or Arnold
          - — advantages over dry heat
          - — simple methods of securing these conditions
            - — wash boiler
              - — top with slant or openings to prevent drip
            - — attachment to steam-heating system often possible
          - — fractional sterilization
            - — advantages
            - — present uses
        - (4) at temperatures above 100° C.
          - — secured by
            - — salts in water
            - — oil baths, etc.
          - — secured by heating under pressure
            - — autoclave; "pressure boiler"
            - — terms describing pressure temperatures: live steam, saturated, superheated, and dry steam
  - c. low temperatures

- (a) freezing
  - 1. result of consequent expansion within the cell
  - 2. effect of ice splinters on cells
  - 3. relation to concentration of salts and osmotic pressure
  - 4. survival period in ice
- (b) temperatures below freezing
  - 1. how obtained
  - 2. results
  - 3. reasons for slight germicidal effect
    - (1) dry cold
    - (2) relation of H_2O to coagulation temperatures

(I) Chemicals used in disinfection, etc.

- a. effect on bacteria
- b. efficiency increased
  - (a) increasing concentration
  - (b) raising temperature — compare law of chemical action
  - (c) adding water (except to watery solutions)
    - 1. relation to metabolic processes
    - 2. makes dehydration more rapid (compare 90% alcohol and absolute alcohol)
    - 3. holds volatile substances favoring chemical combinations
    - 4. adds to penetrating power
      - (1) of heat
      - (2) of gases, as formalin gas
  - (d) combining two chemicals
    - 1. one may facilitate absorption or increase chemical action of the other
    - 2. one may act on the other, liberating a third effective factor, as heat, oxygen
- c. chemicals used. See demonstration for details of method of application, comparative values, etc., of the more important chemicals in the following main classes:
  - (a) acids — advantages and disadvantages of mineral acids
  - (b) alkalies — importance of hydroxyl in such unstable alkalies as lime
  - (c) metallic salts
  - (d) alcohols
    - 1. easily applied
    - 2. rapid action
    - 3. compare drying effect on tissues
    - 4. compare lower per cents with regard to germicidal values
  - (e) benzine alcohols: coal tar disinfectants: carbolic acid, phenol, cresol, etc.
    - 1. high coefficient disinfectants
    - 2. commercial importance
    - 3. soap as vehicle
    - 4. numerous advantages of this group
  - (f) gases (not a related group as here considered)
    - 1. oxygen
      - (1) affects by absence or presence:
      - (2) presence — oxidizes bacteria or food substances
        — affects cell metabolism, energy production

— disadvantage to anaerobic organisms
  — anaerobic a relative term only
  — compare haemoglobin in sensitivity
— relation to spore formation.

2. CO_2 — less effect than generally supposed
3. chlorine — advantages
   — recent revival (water treatment)
4. formalin — advantages as gas disinfectant

(g) essential oils
  1. uses of thymol, oil of cloves
  2. uses of turpentine

C. General discussion of disinfection
  (A) Value of disinfectant depends on
    a. cost
    b. effect upon containers, textiles
    c. stability of action or compounds formed
    d. relative attraction for other albuminous substances
    e. risk of handling
      (a) hands, tissues to which applied
      (b) methods of avoiding mistakes
        1. harmless dyes added
        2. distinctive shapes for containers or for tablets of substance
  (B) Present attitude toward house and room disinfection
    a. compare hospital practice (Chapin)
       — relative dangers: direct contact and air transfer
    b. relative dangers from room during and after convalescent period
    c. methods of house and city fumigation
      (a) simplest methods
      (b) efficiency tested
        1. chemicals used
        2. control on attendant or official
      (c) present practice in N. Y. City
  (C) Present methods of treating wounds
    a. by irrigation
      (a) sterile water
      (b) chemical solutions, e.g., Carrel-Dakin solution
    b. microscopic determination of wound condition

DEMONSTRATION FOR EFFECTS OF PHYSICAL AND CHEMICAL AGENTS

Dry Heat. 1. This ordinary gas oven may be used as a hot air sterilizer (see illustration) for sterilizing test tubes, Petri dishes, etc. The latter has better insulation (an asbestos covering and an air jacket). For sterilizing such materials we use the autoclave. It costs less (for gas), does not char plugs, etc., and does not need to be watched so carefully, nor for so long a time. 150-170 C. for 1 to 2 hours is commonly used for sterilizing glassware in a hot air sterilizer.

Moist Heat — not under pressure. 1. Two types of Arnold sterilizers, both of which use streaming steam, 100° C. The lower pan has a closed chamber for water, causing it to heat more rapidly. The steam rises through the perforated base of the upper chamber. What finally becomes of it?

The more recent ones have this extra cylinder, a cover, which makes an air space one to two inches wide around the inner steam chamber. What effect does this cover have on the temperature inside the sterilizer?

2. In some laboratories where the type of heating system makes it possible, streaming steam is secured by connecting the steam pipes with a copper boiler, with an outgoing pipe for condensation water, much as in the case of steam radiators. That is an excellent system giving quick service most of the working year and costing much less in large laboratories than running individual Arnold sterilizers. See illustration.

3. In our laboratory we use an improvised streaming-steam sterilizer (for sterilizing special media, killing old cultures, etc.) It is a tin ham boiler with a copper bottom. The one now in use has been used almost daily since September, 1913, and is still intact. Its original cost was $1.80, half that of an Arnold sterilizer; this has twice the sterilizing space. Another count against the Arnold sterilizers is that the inner water chamber cannot be dried thoroughly; the cheaper tin ones usually rust out within a year.

Moist Heat — steam under pressure. 1. Autoclave of an early type composed of a single cylinder or jacket. It has a circular door shutting from the inside. The greater the inner pressure, the more firmly the door is pressed against the outer rim; it locks itself, therefore, *auto clave*. Steam may be generated from the water in the bottom of an autoclave, or steam may be piped into the autoclave from a separate but attached boiler, or from the heating system of the building itself.

2. Our autoclave is the single jacket type. Where is the steam generated? How is the autoclave kept closed against the increased pressure of 15 lbs.? Explain the action and value of the safety valve.

Autoclaves should not be opened until the pressure drops to zero (ordinary atmospheric pressure). What may be the effect of opening it before that point is reached?

3. Autoclave consisting of an outer jacket (A) in which moist steam circulates, while the steam in the inner chamber is superheated and rendered *drier*. Sometimes in our single jacket type you will find water of condensation on and in the sterilized glassware; it often leaves the cotton plugs very wet. Note the steam generator (B) attached below the autoclave.

4. An interesting application of the autoclave to household use — the pressure boiler. This is practically like our single jacket autoclave. The shape of the base makes it possible to use this boiler with any form of stove (gas, wood, coal). (See circular.) Their chief value lies in the time reduction in the slow cooking processes usually accorded beans, meats, grains, etc. The small size makes canning a very slow process. There are two advantages, however: (1) the shorter period does not produce the "mushiness" of the discontinuous method of sterilizing; and (2) the higher temperature kills the more resistant spores which escape in other methods.

On this table are a few recent pamphlets dealing with other factors discussed in the lectures and in the reference readings. It is, however, of interest to see for one's self some of the reports of the investigations on which our text-books are based. Look through these, noting marked passages, conclusions, etc.

Pressure. 1. The Effect of Pressure on Certain Micro-organisms Encountered in the Preservation of Fruits and Vegetables, Hite, Giddings, and Weakley (Exp. Station, Morgantown, W. Va., Bull. 146, 1914). Compare the effects upon bacteria and upon the enzymes in the fruit tissues or cells; how does this affect the value of pressure in the canning industry?

Drying

1. The Effect of Drying upon the Viability of Bacteria, Winslow and Abramson (Proc. Soc. Exper. Biol. & Med., 9: p. 107, 1912). What is the 24 hour reduction in *B. coli* dried in sand? Would this rate apply to all organisms: (1) those forming spores? (2) those enveloped in mucus? How would atmospheric conditions (humidity, sunlight, etc.) affect the rate?

Low Temperatures

Germicidal effect of freezing temperatures, etc., (Science, p. 877, June 6, 1913; (p. 770, Nov. 26, 1915). Criticize the theories advanced to explain the relative survival periods in frozen foods and in water; in soft and hard ice.

Light. 1. Absorption of Light by Water Containing Strongly Hydrated Salts. How does this affect the germicidal effect of sunlight on bacteria in water?

2. A Study of the Germicidal Action of Ultra-Violet Rays, Houghton and Davis (Parke, Davis Co., Reprint No. 56, 1914). What are the theories concerning the cause of the germicidal effect of ultra-violet light?

3. Recent Studies of the Biological Effects of Radioactivity, Richards (Science, Sept. 3, 1915). What are the (1) direct and (2) indirect dangers attending the use of radio-active substances in treating human tissues?

From the following part of this demonstration and your reading select disinfectants for 10 different, practical conditions, such as — cut finger, diphtheria dishes, typhoid excreta, swimming pool. Hand in in list form one week from today.

Chemicals

1. Alcohol 95% and 50 % kill *B. coli* in one minute. Why do most physicians prefer 50% for "the field of operation"?

2. H_2O_2, hydrogen peroxide: this acts by liberating O, leaving H_2O. It is valuable (when fresh only) for (1) destroying bacteria, and (2) eating out ("proud flesh") decaying tissue. To insure destruction of bacteria the H_2O_2 must come in contact with them. Therefore: (1) clean away from a wound all blood, pus, etc.; (2) add H_2O_2 in excess until the wound is clean.

3. Aniline dyes have a germicidal effect: (1) because of their chemical affinity for proteins, (that is why they stain cell substances); and (2) because of the alcohol, carbolic acid, etc., used in making the stains; (e.g., carbol-fuchsin is 18% alcohol; the other 82% of it was 25% solution of carbolic acid, making it over 4% carbolic). Such stains as methylene blue and gentian violet are sometimes used in treating wounds; other aniline dyes are injected into the blood system to cure certain diseases, mainly protozoan.

4. Sulphur — used mainly as a gas — (obtained by burning powdered sulphur or sulphur candles); 4-5 pounds per 1000 cu. feet of space.
  — less used now than formerly
    — injures some colors and fabrics
    — has little penetrating power

5. Chloroform ($CHCl_3$) — 1% kills vegetative forms; no effect on spores
  — useful mainly as an antiseptic
    — on wounds
    — liquids (serum, anti-toxin, etc.) that cannot be sterilized by heat

6. Iodoform (CHI_3) — not germicidal
  — but in decomposing tissue makes soluble iodine compounds that may
    (1) act on bacteria
    (2) combine with poisons they have produced

7. Iodine. Surgeons differ greatly about the value of iodine. The accompanying clipping enumerates several of its good points: cheapness, ease with which it can be procured, small bulk, absence of the usual irritation in treated wounds. (Iodine stains are easily removed by applying starch.)

8. Acids.— 4% solutions of most acids are germicidal; antiseptic at least.
  — weaker solutions of some valuable; typhoid killed by
    — sulphuric 1 : 500
    — hydrochloric 1 : 150
  — most acids have too much action on fabrics and metals, for general use.

— boric acid, antiseptic value
— acetic acid is at present interesting investigators; see attached note on vinegar and acetic acid

9. Essences or Essential oils. Most of these have pleasant odors; an advantage? Explain.

Thymol — purchased as crystals or oil
— most useful of these oils — 1 : 1,000 has antiseptic value
— used for envelope moisteners, sponge dishes
— usually rated ¼ as strong as carbolic
— inexpensive

Oil of cloves.

Too sticky for the varied applications of thymol; also too expensive. Often used in dentistry.

10. Silver compounds:

Argyrol — silver and albumin compound. It deteriorates rather rapidly. Its entire lack of caustic properties — one of its chief values — makes it difficult to be sure of its integrity. It is valuable for cuts, scratches, etc.; and some find it very helpful for sore throats. Apply (10-25%) with a brush. (Argyrol stains are easily removed with water or salt and water.)

Silver nitrate — too expensive for general use. Used for local treatment — infected eyes, throats — but too caustic to be handled by any except doctors or nurses.

11. Other metallic salts or compounds:

a. Corrosive sublimate (poisonous)
— water solution, saturate — 1 : 16
— alcoholic solution, a stronger disinfectant — why? (two reasons).
Usually sold in H_2O solutions
1 : 100,000, inhibits most bacteria
1 : 50,000, kills many
1 : 1,000, good germicide

Alcoholic solution 1 : 500 in 95% alcohol. This is a little too "drying" for constant application, but excellent for securing aseptic conditions, as in cuts. All such poisons should be colored with some non-irritating coloring to prevent accidental poisoning. This contains methylene blue — a few drops to a half pint.

b. Platt's chlorides — See label for the salts contained in this mixture. These are not volatile; the value of this preparation depends, therefore, on liquid application only. This is used in dilutions of 1 : 4. How does it compare in price with other liquid disinfectants?

Advertisements of such patented, commercial disinfectants are sometimes misleading; e.g., one reads, "Place a little of ——— in a dish on the radiator and avoid all disease."

c. Copper sulphate — valuable as a disinfectant for water
— now superseded by hypochlorites and possibly by chlorine (see water)
— 1 : 400,000 destroys typhoid — in 24 hours
— 1 : 1,000,000 destroys malodorous algae (not uncommon in reservoirs late summer)
— not injurious to people in above strength

12. Sodium compounds:

a. soap suds — slight germicidal value
b. sodium carbonate — 5% — kills vegetative bacteria in a few minutes
c. sodium bicarbonate — no bactericidal effect; popularly used to counteract the acidity of the stomach
d. sodium hydroxide — 4% kills resistant spores in one hour
— too great action on human skin to be generally used
e. sodium bypochlorite, calcium hypochlorite or a mixture of both are used as a disinfectant for wounds, for treating water, etc. The

accompanying account of the Carrel-Dakin method will be interesting to nurses; it is used a great deal in the war hospitals at the front; hypochlorites and chlorine in relation to water will be treated under the topic Water.

13. Formalin. The disinfectants on this table are made of or combined with formalin. Formalin is rarely used as a liquid disinfectant. It is too caustic, and is "very hard on the hands." As a gas disinfectant it is in practical use equalled only by chlorine; it does not have the undesirable bleaching qualities of chlorine (e.g., clothing, upholstering).

— advantages — little effect on fabrics and metals
  — true deodorizer
— disadvantages — very irritating to mucous membranes, and but little effect upon insect pests; this makes many doubt its efficiency
— efficiency readily increased by heat and by water vapor

a. The preferred methods of using formalin therefore include some way of vaporizing it —

(a) allowing it to evaporate slowly from a wet surface, as a sheet, or preferably
(b) using heat
  1. burning formalin candles
  2. heating the formalin with a flame (gas, etc.) as in Shering's Lamp and Disinfector;
  3. heating the formalin by chemically generated heat
    (1) by adding water and formalin to unslaked lime. The slaking of lime generates sufficient heat to vaporize the formalin.
    (2) by mixing paraformaldehyde (a powder form) with potassium permanganate and water.

This combination acts slowly, giving the operator time to leave the room. An added value of this disinfectant is that it liberates O. The heat adds to the value of the disinfectant; the vaporized water, to its penetrating power.

See Munson's disinfector. This does not need to be set up in the room, but the generated gases can be let in through the keyhole.

b. Formalin tablets said to give off enough formalin vapor to prevent bacteria from developing. Some recommend them highly at the beginning stage of sore throat. It seems very probable that sufficient formalin to make them effective would be irritating to the mucus membranes.

c. Room disinfection: 10 oz. formalin to every 1000 cu. ft. See Report of N. Y. Research Laboratory, 1912, for approved details of a quick and efficient method.*

As indicated in the lectures, opinion has veered rapidly with regard to the values of disinfection. It was stopped in part of New York City (1914) but continued in one borough, Brooklyn. After a period of several months, the results did not seem to justify its continuance, so official disinfection has been discontinued for the entire city, except following smallpox and where people ask for it.

See official announcement concerning this.

*An ingenious check on the disinfection had been established — which is itself still of interest. This consisted in drying bouillon cultures of *B. pyocyaneus* on sterile threads. *B pyocyaneus* is considered about as resistant as most pathogenic organisms. These bacteria-covered threads were then dried and placed in these thin envelopes which the operator affixed to the wall of the room to be disinfected. (These envelopes bear data concerning the driver, room disinfected, etc.). When the operator returned to the house to open the disinfected room, he secured this envelope and returned it to the city laboratory. The thread was then placed into a test tube of bouillon and incubated. If the green-pigment organisms on it had been killed, no color appeared in the test tube. Green color would indicate incomplete disinfection, for which the health officer or the occupant of the house was responsible; in such cases the operator would be sent back to complete the work.

14. Carbolic acid and the high coefficient disinfectants.

a. Crude carbolic — in liquid form. Not easy to bring into true solution.

b. Diluted carbolic; less attractive than the pure, not suitable for treating dress-goods, etc. (too oily), but used for utensils, excreta. The present European war has so affected the price of carbolic acid, that substitutes are in demand. See description of Edison's recent work on carbolic acid.

c. Pure carbolic crystals — (C_6H_5OH) soluble in warm water — used for staining (mordant) and as a disinfectant. Its efficiency is greatly increased by a little acid, such as HCl. Compute present cost per gallon — 5% solution.

Advantages of carbolic: does not destroy fabrics and colors
does not combine readily with albuminous substances (as excreta)
efficient in low % 1:400 kills most regular forms;
1:20 kills most spores in a few hours.

d. Lysol, 2 times as strong as carbolic (half cresol). Sold in solutions and therefore popular.

e. Cresol ($C_6H_4CH_3OH$).

Stronger than lysol (4 times as strong as carbolic).

Often combined with potassium soaps; then 2 times as strong as carbolic.

f. Creolin — Pearson. See circular.

A cheap disinfectant; purchased in solutions; a dollar bottle makes 25-50 gallons of disinfectant.

g. Cresol, lysol, etc., are often called high-coefficient disinfectants. For example, lysol, as rated above (twice as strong as carbolic), would be given a coefficient of 2; cresol, of 4.

h. C. N. is another popular high-coefficient disinfectant. A twenty-five cent, ½ pint, bottle makes 3-8 gallons of disinfectant. C. N. is advertised as 5-6 times as strong as carbolic.

Compare the results (chart) obtained with C. N. and phenol in the test made by one of our students. At that rate what dilution might be used? How would its cost compare with the estimate just given? (All reputable houses make very conservative estimates of germicidal value; what connection between this fact and the pure drug laws?)

i. Neko (marked circular). One of the newer disinfectants — with a coefficient of at least 16-20.

j. Other high coefficient disinfectants now on the market: Hycol, chinisol, etc. Make sure always (1) that the strength or coefficient is definitely stated and (2) that the preparation comes from a well-known firm or is vouched for by a reliable authority.

k. Disinfection (Parke, Davis & Co., Reprint No. 60, 1914). Read the marked passages for a comparison of the high-coefficient disinfectants. Compare the good points of carbolic with those enumerated on p. 109.

l. Facts and Fallacies in Disinfection, Hamilton (Parke, Davis & Co., Reprint No. 121, 1917). A discussion of various disinfectants.

m. A Hand Book of Antiseptics, Dakin and Dunham (Macmillan, 1917). Designed especially for those (surgeons, nurses) caring for the wounded.

n. To test the strength of a disinfectant it is made up into solutions of various strengths. To definite amounts of these solutions are added small but definite amounts of bacteria (a loop, 1 c.c., etc.). After designated intervals (2½ minutes, 10 minutes, etc.) a small amount of this mixture (bacteria and disinfectant) is transferred to a sterile nutrient (bouillon usually). These are incubated for 24 hours or more. If growth of the inoculated organism occurs, we know that that dilution of the disinfectant was not strong enough.

(Sometimes pigment-forming organisms are used; the color of the tube then indicates whether the growth is due to the original inoculated organism; it lessens the routine work; how much?)

o. These pamphlets describe more definitely the method of comparing the strength of different disinfectants; look through if interested. (a) Method of Standardizing Disinfectants With and Without Organic Matter, Anderson and McClintic; (b) The Determination of the Phenol coefficient of Some Commercial Disinfectants, McClintic. Both from Hyg. Lab., Bull. 82, 1912. (c) Standardization of Disinfectants, Hamilton and Ohno (Parke, Davis and Co., Reprint No. 45, 1913).

Other practical phases of disinfection

1. In a large town (90,000) two leading drug stores were asked to name the disinfectants they kept for sale, (1913). These had in common:

1. chloride of lime
2. carbolic acid
3. mercuric chloride
4. lead nitrate
5. copper sulphate
6. potassium permanganate
7. iodine
8. formaldehyde
9. hydrogen peroxide
10. sulphur
11. calcium hypochlorite

Do these cover the needs of ordinary living conditions? How do they compare with those available at your drug store?

2. Some Common Disinfectants, Dorset, (Farm. Bull. 345, 1908).
What would you add to this today (1918)?

3. Other helpful government pamphlets are shown here; read at least one.
a. Practical Methods of Disinfecting Stables, Pope, (Farm. Bull. 480, 1912).
b. The Sanitary Privy, Stiles, (Farm. Bull. 463, 1911).

4. This soap advertisement has a "patter" of bacteriological terms. Does this statement concerning bacteria on soap follow from the previous sentence? Is it supported by experimental evidence?

Note that this is an attempt to fool "all the people" (1) through their partial knowledge of bacteriology, and (2) by an abundance of technical terms. That it is not the result of definite experimental work or accurate knowledge is shown when they use *antiseptic* in the sense of disinfectant; why is this difference important?

5. Find in the newspapers, on bottled preparations, packages, one advertisement which does not seem trustworthy; give the advertisement and your reason here.

EFFECTS OF PHYSICAL AND CHEMICAL DIFFERENCES COMMON IN LABORATORY PRACTICE

1. Inoculate *B. prodigiosus* as indicated below.
   (a) 2 slants on peptone agar (made as usual with meat extract and peptone).
   (b) 1 slant on agar containing dextrose but not peptone.
   (c) 1 stab into a straight agar tube (made with meat extract and peptone).

2. Incubate all at room temperature except one of the meat extract-peptone-agar slants, which is to be incubated at 37°.

3. Compare next lesson. Considering abundant growth and *red* pigment as normal characteristics, what conclusions do you draw concerning (1) the relation of sugar and of peptone to normality and vigor of growth? (2) the relation of oxygen? (3) the effect of temperature?

OTHER EXERCISES IN GENERAL BACTERIOLOGICAL TECHNIQUE

1. **Isolation of pure cultures.** a. "Fish" a distinct or well-separated colony from one of the air-exposed plates to broth (as shown). Incubate at room temperature for 1 day. If no apparent growth (gross appearance), incubate for the time remaining before your next lesson at 37°.

b. Examine a smear at the following class session for purity.
c. Further evidence of purity may be obtained by streaking a loopful of broth on a sterile agar plate (as shown). Many bacteriologists prefer making a pour plate of the culture suitably diluted. What advantage§ in the pour plate over the streak plate? What difficulty may you encounter?

2. **Isolation of a coccus culture for continuance in broth.** To demonstrate the acquisition of a reasonable amount of technique, the student is asked to isolate a pure culture, carry it through seven successive transfers in broth, demonstrating its purity at each stage, and finally transferring it to slant agar or streaking it on a Petri dish as additional indication of its purity. The final stage on agar may be handed in. Carry this series on as rapidly as your class work permits; twenty-four hour intervals are sufficient, or a week may elapse between transfers.

It will be wise to keep one or two back transfers, for a contamination may unexpectedly be found to run back 1 or two transfers. In that case it will be necessary to go back to the last pure culture, or to re-isolate.

Rods vary so with age that the coccus organisms will be more satisfactory for beginners. The colored colonies on your air plates are quite likely to be cocci or yeasts, either of which may be used.

3. **Distribution of bacteria.** a. Inoculate a tube of broth with material from one of the various places discussed in class: doorknobs, paper money, coins, pencils, finger nails, skirt-braids, gloves, skin, etc. (A convenient method is to invert a tube of broth, thus wetting the inside of the cotton plug. This moist plug can then be rubbed over the skin, knob, etc., replaced, and washed off in the broth in the tube. Incubate at room temperature until the next lesson.)
b. Make a smear of the broth tube inoculated from one of the various sources and keep at room temperature. Dry, and keep from dust until the next lesson.
c. Incubate the tube at 37° until then and make a second smear. Stain both and compare. How may similarity be explained? Differences? How many morphological types do you find?
Which is the most common? Relate the power of spore production to the above.
In what localities, substances, etc., did you fail to obtain cultures?
What bearing have your results upon health and disease?

4. **Examination of sputum, etc.** a. In such examinations, one usually has in mind a definite organism, e.g., diphtheria in suspected sore throats, typhoid in fever patients, or tuberculosis with certain clinical symptoms. The methods of conducting such examinations are mainly three.

(a) Direct microscopic examination of sputum or other materials, often with special stains, e.g., methylene blue shows up the peculiar unequally staining character of diphtheria cells.
(b) Implanting of the sample in special media, e.g., diphtheria on blood-serum media, where it usually outgrows other throat organisms.
(c) Streaking out (or plating out) of a small amount of the sample on a Petri dish of plain agar, or of selective media, as Conradi-Drigalski agar (lactose and litmus and crystal-violet) for *B coli*, or "brilliant green" agar for typhoid. This plate is then incubated, and from it characteristic colonies are selected for microscopic or cultural verification.

b. Streak out, on a plain agar plate, a throat swab rubbed over the tonsils, or some saliva collected and diluted in a tube of broth. Incubate 1 day (or longer if neces-

sary) and then "fish" to separate tubes of broth, as shown, a few of the more delicate, semi-transparent colonies. These will probably be streptococci. Examine your fishings for purity 1-2 days later. If not pure streptococci, what is the next step?

X. BACTERIA IN AIR

(Brief; much belonging under this topic is covered elsewhere, e.g., disinfection of rooms, and effects of light)

Illustrative material: Charts giving bacterial counts for various localities and conditions

A. Number affected by
    (A) Agents already discussed in IX
    (B) Altitude
        a. Pasteur's flask experiments
        b. counts of other investigators
    (C) Number of inhabitants
    (D) Suspended particles in air (dust, lint)
        a. proportion of bacteria to dust particles
        b. explanation of bacteria *in* hailstones
    (E) Water surface exposed
        a. relative counts on land and lakes, oceans, glaciers
        b. tests of sewer air
    (F) Washing effect of snow and rain
B. Methods of estimating number
    (A) Exposure of agar plates to air
        — effect of air currents
    (B) Plates made by filtering or washing given volumes of air
        a. centrifuged washings used
        b. precipitate used — filtered through sand, sugar
        c. washings filtered through melted agar
C. Relation to ventilation, including recent findings of Ventilation Committee
    (A) Crowd poisons?
    (B) CO_2; compare washed air experiments
    (C) Humidity
    (D) Radiation and conduction of heat
        a. skin as thermal regulator
        b. dress
        c. artificial aids; heating and cooling devices
    (E) Other physiological factors
        a. odors
        b. appetite?
        c. effect of heat on relation of oxygen and haemoglobin
    (F) Relation of crowded cars and rooms to health
        a. opportunities for direct transfer of disease organisms
        b. recent rulings of N. Y. City Board of Health

AIR PLATES

Usually made early in the course. Selections from the whole class form the material for p. 37, no. 18.

1. Make an agar plate, as shown. If the agar does not cover the whole plate, a slight horizontal movement back and forth while still resting on the table will spread it evenly. Do not pick it up to accomplish this; why?

2. When "set," expose, by removing the cover, for 5 minutes in the room or place selected. Try to arrange with a fellow student for paired situations: a room before and during dusting; near a closed and an open elevator shaft; or out and in doors at the same level.

3. Place your exposed plate in the tray so designated, and the plate will be incubated at 37° for 2 days, and at room temperature for the rest of the time elapsing before your next class hour. What advantage in using both temperatures?

4. Record on the chart provided the colony count (molds, bacteria) on your air-exposed plate. Compare with the other counts recorded. Can you explain the relative numbers obtained in the various places or localities? What two factors seem to be most influential?

DEMONSTRATION ON BACTERIA IN AIR

1. Photographs of three agar plates exposed for the same length of time during dusting operations on the same day. (1) Feather duster; (2) dry cloth; (3) damp cloth. Explain.

2. Sweeping experiment (as above) with dry broom, damp broom, and vacuum cleaner.

References on Bacteria in Air* (See marked passages.)

1. The Problem of City Dust, Bolton (Trans. Am. Soc. Heating & Ventilating Engineers, 1915).
2. A Quantitative Study of the Bacteria in City Dust (etc.), Winslow and Kligler, (Am. Journ. Public Health, 2, p. 663, 1912).
3. The Microbic Content of Indoor and Outdoor Air, Winslow & Brown, (Monthly Weather Review, p. 452, July, 1914).
4. Fresh Air, Lee, (Popular Science Monthly, p. 313, April, 1914).
5. Standards of Ventilation in the Light of Recent Research, Winslow, (Science, p. 625, April 30, 1915).
6. Ventilation of different types of buildings:
   a. (1) The Heating and Ventilation of School Buildings, Kimball.
      (2) Minimum Sanitary Requirements for Rural Schools, (Press of American Medical Association, Chicago, 1914).
      (3) The Part Played by Temperature in School Room Ventilation, Palmer, (School and Society, Vol. 2, p. 55, 1915).
   b. Church Ventilation, Kimball, (American Churches, Vol. 1, published by The American Architect, N. Y. City).
   c. Hospital Ventilation from the Point of View of the Clinician, Miller, (Journ. Am. Med. Assn. Vol. 63, p. 1623, 1914).
7. Various reports of the New York State Commission on Ventilation.

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*This and all similar lists of pamphlets and references are not final. Such lists vary from year to year with the articles and abstracts available. Those listed in this outline are placed first on the table; those apparently lacking have been replaced by more recent and more helpful ones, to be found in this same exhibit.

XI. BACTERIA OF WATER

A. Relation to disease
    (A) Special diseases: typhoid, cholera, dysentery
    (B) General health: Mills — Reincke theory?
B. Origin of water bacteria
    (A) Air — winds, rain, snow

- (B) Animal excretions (including sewage)
- (C) Plant and animal decay
  - —burials (earth as filter)
- (D) Organisms persisting in soil
  - —tetanus and anthrax; survival records

C. Control of water pollution
- (A) Wells and springs
  - a. relative position of barns, outhouses, etc.
  - b. surface slope
  - c. underground strata and drainage
- (B) Community reservoirs and watersheds and their protection

D. Bacteria in ice
- (A) Effect of freezing on typhoid, etc. (current theory of seasonal typhoid)
- (B) Variation in effects produced by slush and hard ice
- (C) Comparative values of artificial and natural ice
  - a. original bacterial content
  - b. age in relation to bacterial content
- (D) The use of ice *in* water and foods

E. Methods of improving water supply
- (A) Predatory organisms; difficult to measure results
- (B) Storage
  - a. sedimentation
  - b. exhaustion of food supply
- (C) Light
  - a. sunlight
    - (b) depth limits
    - (b) modified by
      - 1. turbidity
      - 2. salt content (?)
      - 3. rate of flow
  - b. ultra-violet light
    - (a) modified also by salts in solution
    - (b) practical applications
- (D) Oxidation—natural (see oxygen below)
- (E) Dilution
  - a. often relied upon
    - (a) city water supplies
    - (b) swimming pools
  - b. aided by effects of
    - (a) light
    - (b) aeration
- (F) Filtration
  - a. soil as natural filter
    - (a) flora of upper 6 inches
    - (b) flora 4-5 feet below surface
  - b. artificial filters
    - (a) city filters
      - 1. sand
      - 2. improved by
        - (1) coagulatant in water
        - (2) gelatinous surface on sand bed
      - 3. effect of slow sand filter on bacterial count
      - 4. possible rates of treating large supplies
    - (b) house filters
      - 1. dangers of
      - 2. best types
      - 3. emergency filters

            - 4. care of house filters: cleaning; reversible types
- (G) Heat
    - a. boiling
        - (a) death points of ordinary pathogenic bacteria
        - (b) subsequent aeration to make palatable
    - b. application of lower temperature for large supplies
        - (a) advantages: cheap, and continuous application possible
- (H) Distillation
    - a. comparison of distilled and boiled water
        - (a) bacterial content
        - (b) mineral content
            - 1. minerals added for drinking water
            - 2. aeration for taste, sparkle
- (I) Chemical agents
    - a. natural
        - (a) light (already discussed)
        - (b) oxygen
            - 1. large bodies of water
            - 2. effect of broken surface: brooks, waterfalls
        - (c) popular misconceptions concerning "self purification"
    - b. artificial methods
        - (a) copper sulphate — effect on bacteria and on algae
        - (b) hypochlorites
            - 1. often confused with chlorides
            - 2. oxygen and chlorine liberated
        - (c) chlorine — present views of value

F. Examination of water

- (A) Bacterial count
    - a. number low
        - (a) unless excess organic matter present
        - (b) unless pollution recent
    - b. accepted standards
        - (a) spring, well
        - (b) river
        - (c) lake
        - (d) city supply
    - c. importance of method of taking sample
        - (a) size of sample
        - (b) depth, distance from shore and from sewers
        - (c) age and care of sample
        - (d) source and history of supply
- (B) Kind more important than number
    - a. determined by
        - (a) search for pathogenic organisms themselves
        - (b) demonstration of accompanying organism from fecal or other animal sources
    - b. methods
        - (a) microscopic demonstration of streptococci
        - (b) presumptive tests for — coli, typhoid, and cholera
            - 1. based on characteristic growth
                - (1) special nutrients in agar (cholera, coli)
                - (2) with dyes sensitive to acids, alkalies, etc. (coli, typhoid)
            - 2. applied water, oysters, milk products
        - (c) chemical tests
            - 1. based mainly on organic matter content

2. tested mainly by
   (1) presence of nitrites and ammonia
   (2) amount and kind of gas formed
   (3) amount of O absorbed

WATER EXAMINATION — BACTERIAL COUNT

1. a. Make two water plates (as shown) using (1) the original water sample and (2) a 1/10 dilution. Use 1 c.c. of each to make the agar plate. The agar should be melted, cooled, and kept at 42°-45° until ready for use. (Why not a lower temperature? higher?) Incubate at 37° for 2 days.

b. Count with a lens the colonies that have developed. (Why with a lens?) How many bacteria per c.c. are indicated by each plate? Record on the charts provided. How do your results compare with those obtained by the rest of the class? Give possible explanations.

2. a. With the above make also an agar plate of filtered water, catching your sample from the running tap which has the Berkefeld filter attached. This filter has been in use for the number of hours indicated by the tag.

b. Compare with the bacterial count of the unfiltered water. Could you explain a *higher* bacterial count for filtered water?

c. Compare the number per c.c. with the count made by the other sections. How does time affect the value of house filters?

WATER DEMONSTRATION

(Review chemical disinfectants, also, in this connection; new material on that topic is included here.)

Organisms associated with undesirable or dangerous water conditions

1. Agar slants of *Bacillus coli* and *Streptococcus,* and the organisms causing typhoid and cholera. Under the microscopes placed near the respective slants are stained slides of these organisms.

(a) *Streptococcus,* a long-chained form. Some strains are always short-chained, 4-8 organisms only. Long-chained ones are probably never found in water free from animal pollution.

(b) *Bacillus typhosus.* Notice the range in size. This is partly due to the fact that some of the bacilli remain connected after division, forming filaments.

(c) *B. coli.* Could you be sure of distinguishing between these and the typhoid organisms? Often found in water; almost invariably present in feces and sewage.

(d) *Vibrio cholerae* or cholera. Short, slightly curved organisms, often comma-shaped.

Water examination

1. Plates made from tap water. 1 c.c., 1/10 c.c., and 1/100 c.c. to a plate. Are the respective colony counts what you would expect? Explain the general larger size of the colonies in the weaker dilutions?

What does a large count indicate? Why? Why do we not depend on number only in judging water?

2. Streptococci, if numerous enough, may be identified in water by direct examination. (This test is uncertain except in warm countries; why?) Are any present in this stained slide from the locality indicated?

3. Other organisms are tested for by presumptive tests. Bile, neutral-red, lactose, and litmus are among the special substances used. Note the action of typhoid and of *B. coli* on these litmus-lactose-agar plates. Why do we examine for the comparatively harmless *B. coli* instead of typhoid? Are we justified in condemning water on positive *B. coli* tests?

4. Plates of three dilutions (1:10; 1:100; 1:1000), of the same sample of water on lactose-litmus-agar plates; these show *B. coli* in the 1/10 dilution plate only. What does this indicate concerning the condition of the water? In New York City 10 to 50 *B. coli* colonies per c.c. are allowed in oyster tests; not more than 10 in drinking water. Is this reasonable?

Warning. Do not hold any *B. coli* plates so as to get any of the condensation water on your hands. What should be done with hands, tables, etc., accidentally so moistened?

5. Agar plate made from a water-supply tank. How does the count compare with the plate (same dilution) made from tap in the basement—the same water that later fills the tank? Explain. Would the opposite ever be found? Why, or under what conditions?

6. Sterile broth containing neutral-red dye and lactose. Compare with the next tube, in which acid-forming bacteria are growing.

7. These tubes contain *B. coli* growing in neutral-red and lactose broth, 1, 2, and 3 days respectively. How does *B. coli* affect this medium? Does time enter into the reaction?

8. Water from the source indicated by the accompanying label was inoculated into this tube of neutral-red. Did it contain *B. coli?*

9. A little oyster liquid (1 c.c.) was added to this tube of lactose neutral-red broth. Did it contain *B. coli?*

10. Bile and lactose broth. Bile is considered to have germicidal or antiseptic qualities for most organisms. Would you expect *B. coli* or other intestinal organisms to be sentitive to (or inhibited by) intestinal secretions of this kind? Is *B. coli* presumably present in the water sample incubated in this tube? (Bile is less in favor now for *B. coli* diagnosis than formerly.)

11. The Standard Methods of Water Analysis is the book followed by the leading laboratories in the country. It includes:

(1) collecting water samples (p. 1-3)
(2) physical examination: color, sediment, etc. (p. 4-13)
(3) chemical examination (p. 14-70)
(4) bacteriological examination and a description of the common water bacteria (p. 77-137).

Glance through to get a general idea of these four parts. What media are used? Is the emphasis on bacterial counts? on presumptive tests?

Filters

1. Berkefeld, Pasteur, and Chamberland filters. Booklets showing styles: simple, reversible, pressure filters, etc.

2. This house filter (Berkefeld) has been unscrewed to show the various parts. How many possible places for leakage? How may such leakage be prevented?

3. Home-made filters. Compare these home-made filters with the descriptions marked in Park (chap. on water).

a. Flowerpot-sand filter.
b. A home-made cotton filter. Note the wire support for the cotton pad. A common lamp chimney would do very well; too large a vessel allows too much weight to press upon the cotton sieve, which might break it.

4. A cheap commercial cotton filter. These cotton pads should be replaced each morning. Why? For ordinary home use such a simple cotton filter will remove sediment, etc.; and boiling the water will make it bacteriologically safe. What dangers attending the use of the ordinary house filter are avoided in this method?

Chemical Treatment of Water

1. What methods of rendering drinking water safe were given in the demonstration on Effects of Physical and Chemical Agents?

2. Copper sulphate. The Effect of Copper upon Water Bacteria, Kellerman & Beckwith, (Bull. 100, Part 7, Bureau Plt. Ind., 1906).

3. Sterilization of Niagara Falls Water by Ultra-violet Rays, (Engineering Record, June 20, 1914).

4. Hypochlorites.
    - a. The hypochlorites so widely advertised today are *not* chlorides.
        Calcium chloride is $CaCl_2$
        Calcium hypochlorite is $CaCl_2O_2$. It has O to liberate, this gives it disinfecting (oxidizing) value. Chlorine is also liberated and adds to the disinfecting power of the hypochlorite.

HOCl is present, as hypochlorous acid, which liberates O also, forming HCl; HCl is also a disinfectant, though usually too small in amount in such cases to be of real value.

Sodium and calcium hypochlorites are both used in water disinfection. They are called bleaching powder, the usual proportions are 1 or 1½ parts to a million parts of water.

- b. Literature dealing with the hypochlorite treatment.
    - (a) Chloride of Lime in Sanitation, Albert H. Hooker, (Wiley, 1913). Does "Chloride" as here used mean chloride or hypochlorite?
    - (b) The Use of Calcium Hypochlorite in Water Purification, C. M. Hilliard, (Proc. Indiana Engineering Society, 1913). Look at the marked passages.
    - (c) Comparative Resistance of Spores and Vegetative Cells of Bacteria toward Calcium Hypochlorite, C. M. Hilliard, (Proc. Soc. Exp. Biol. & Med., 1912). "Available chlorine" implies that the reduction described is due to the chlorine liberated. How does that compare with the earlier statements with regard to the disinfecting power of hypochlorites?

5. Chlorine. Disinfection by Chlorination of Public Water Supplies in New York State, Horton, (Health News, Feb., 1915). Chlorine gas is apparently coming into favor. What are its good points? How does it compare with hypochlorites for emergency treatment, as in a typhoid epidemic?

6. Water Purification and Typhoid Fever Number, (Health News, Feb., 1915).

7. Swimming Pools.
    - a. Chart by Bunker showing the effect of hypochlorites on the pool at Brown University. How quickly do hypochlorites act?
    - b. Studies on the Sanitation of Swimming Pools, (Manheimer, Journal of Infectious Diseases, July, 1914). What method is used in the pools you have used or use now? What diseases are the authorities trying to guard against? Why are *B. coli* tests used in estimating pool conditions?
    - c. Comparison of Methods for Disinfecting Swimming Pools, Manheimer, (Amer. Phys. Education Review, Vol. 22, p. 22, 1917).

References on Water Supplies (and Sewage)

1. Water and Public Health, Fuertes, (Wiley, 1905). Compare the typhoid death rate with the type of water supplied the various cities as listed in the charts following p. 32.
2. Water Purification and Sewage Disposal, Tillmans, (Van Nostrand, 1913).
3. Public Water Supplies, Turneaure & Russell, (Wiley, 1914).
4. Examination of Water, Mason, (Wiley, 1917).
5. Bottled waters.
    - a. Bacteria in Commercial Bottled Waters, by Obst, (U. S. Bull. 369, 1916).
    - b. Sanitary Code, New York City. See marked places.
6. Sewage Disposal, Kinnicutt, Winslow, and Pratt, (Wiley, 1913).

Water and Disease

Numerous pamphlets, e.g., Health News, published by New York State, marked to show relation of water to typhoid, enteritis, etc. These maps and charts have been collected from various books, pamphlets, etc.

XII. MILK

Illustrative materials: Charts showing (1) chemical composition of milk; (2) effect of varying temperatures on the bactericidal action of milk; (3) variations in bacterial counts due to age and temperature; (4) relation between different types of milk and the health of babies.

- A. Milk-borne diseases
  - (A) Connection established
    - a. typhoid
    - b. diphtheria
    - c. scarlet fever — throat secretions apparently dangerous
    - d. tuberculosis
      - (a) human
      - (b) bovine — special danger to children
    - e. septic sore throat
      — recent epidemics
    - f. foot and mouth disease
      - (a) children most susceptible
      - (b) cheese and butter may transfer
    - g. staphylococcus and streptococcus infections
  - (B) Diseases not yet positively traced to milk: mumps, measles, whooping cough, chickenpox, and smallpox
- B. Milk as obtained from healthy cow
  - (A) Food value
    - a. rich in needful nutritive substances
    - b. foods in soluble form
  - (B) Bacteria at time of milking
    - a. fore-milk; relate to
      - (a) structure of teats
      - (b) moist tips of teats
      - (c) contact teats with ground, etc.
    - b. udder
      - (a) persistence of pyogenic invaders
        - 1. actually diseased glands
        - 2. udder not always infected in disease
    - c. adjacent lymph glands
    - d. opportunities for entrance of bacteria
      - (a) cow: saliva, coat, moist teats, tail
      - (b) dust, fecal material in stalls, filthy barnyards
      - (c) air currents during milking
      - (d) milker
        - 1. unwashed hands; saliva
        - 2. clothing — soiled by other barn work
      - (e) pails
        - 1. incompletely cleaned
        - 2. size of open top
      - (f) small animals allowed in stables
  - (C) Freedom from micro-organisms
    - a. mid-milk relatively free, at least in healthy cows
    - b. comparison of samples taken with aseptic precautions
- C. Rapid increase in bacterial content of milk
  - (A) In spite of bactericidal action of fresh milk
    — influence of temperature on bactericidal action
  - (B) Explanations of increase
    - a. foods in milk
      - (a) all nutritive groups represented in milk
        — relate to range in bacterial species found in milk

- (b) suitable proportions
- (c) in solution (ready for absorption)

- b. water present; necessary all metabolic processes
- c. range of favorable temperatures; why cool quickly?
- d. age of milk when it reaches consumer

- (C) Relate to comparative death rates of breast-milk and cow-milk babies

- D. Control of the flora of milk
  - (A) Reasons for keeping bacterial count down
    - a. increase in pathogenic organisms
    - b. increase in non-pathogenic organisms causing undesirable changes in milk
      - (a) ptomaines
      - (b) souring
        - —type of souring organisms important
  - (B) Methods of affecting the bacterial content
    - a. low temperatures
      - (a) effect on growth of bacteria
      - (b) enforced refrigeration of milk supplies
    - b. chemicals
      - (a) kinds used
      - (b) why prohibited
        - 1\. injurious
        - 2\. mask real quality of milk
    - c. partial evaporation (White cross milk)
      - (a) diluted when used
      - (b) keeping qualities related to
        - 1\. temperature used
        - 2\. water content
    - d. boiling — objection — mainly taste
    - e. sterilizing
      - (a) less often used for milk as food
      - (b) effect of higher temperatures on precipitation
      - (c) unnecessary: pathogenic death point lower
    - f. pasteurizing
      - (a) real origin of term
      - (b) alters milk less than boiling and sterilizing
      - (c) temperatures advised
        - 1\. time limit for each
        - 2\. "flash" and "holder" processes
        - 3\. advantages of pasteurizing *in* the bottles
        - 4\. advantages of "capping" by machine
      - (d) relative digestibility
        - 1\. for babies (compare protein "indigestibility")
        - 2\. vitamins
        - 3\. orange juice to replace caseinogen
      - (e) effect upon bacterial flora (also upon white corpuscles; important in distinguishing if milk has been heated)
        - 1\. kills common pathogenic bacteria
        - 2\. does not kill spores
          - (1) compare therefore relative effect on the common protein-breaking and the acid-forming bacteria
          - (2) relate to clean and filthy milk
      - (f) arguments against pasteurization
        - 1\. cannot remove — dirt, odors, accumulated wastes and poisons
        - 2\. delays souring
          - (1) cloak for poor grade of milk

                - (2) permits careless methods of milking, etc.
            - 3. unreliable — imperfect or careless procedures following pasteurization
            - 4. followed by nutritional diseases (scurvy, etc.)
                - (1) not substantiated
                    - — effect of city milk stations
                    - — compare tables on baby feeding
                        - — gains in weight
                        - — disease
                - (2) can be obviated by proper additions to diet (fresh fruit, certain vegetables)

- E. Methods of examining and controlling milk supply
    - (A) Plate method
        - a. reasonable count standards
            - (a) special milk
                - 1. relate to care of cow, milking procedures, etc.
                - 2. extra price justified
                - 3. count less than 100 per c.c.
            - (b) ordinary milk — 6000-12,000 per c.c., if kept iced
        - b. average counts allowed by city boards of health
        - c. incubation (temperature and time) important
    - (B) Direct examination or count
        - a. advantage to consumer
        - b. demands more skilled workers than (A) above
        - c. acceptable technique yet developed?
            - (a) clump versus single bacterium counts
            - (b) adapted for pasteurized milk? (dead bacteria may take stains also)
        - d. also look for
            - (a) special bacteria, chiefly streptococci
            - (b) leucocytes
    - (C) Presumptive tests — special media as with water for *B. coli*
    - (D) Inoculation tests (guinea pig) for pathogenicity; weak points
    - (E) Cleanliness tests
        - a. sieve method — Park and Schroeder
        - b. "dirt" (manure, hair, etc.); associated with high counts
    - (F) Score card system
        - a. includes
            - (a) cow: health, cleanliness
            - (b) stable conditions
            - (c) workers or dairymen
            - (d) cleanliness tests of milk
            - (e) may include bacterial record
        - b. sliding-scale prices paid great incentive
        - c. present regulations for New York City
            - (a) labels
                - 1. grades — a, b, c
                - 2. dates affixed to all bottles
            - (b) requirements for raw and pasteurized grades
                - 1. bacterial limits before and after pasteurization
                - 2. farm and animal inspection
            - (c) meaning of special terms: selected, inspected, guaranteed, certified

F. Milk products
- (A) Microscopic examination worth little in this connection
  - a. presence of mixed organisms easily demonstrated
    - (a) final flora not necessarily causal
    - (b) serial flora; changes not due to single, nor even contemporary, organisms in most cases
- (B) Fermented milks
  - a. alcoholic, e.g., koumiss
  - b. acid
    - (a) common soured milks
      - 1. 1 per cent checks most organisms
      - 2. *B. bulgaricus*
        - (1) active up to 4 per cent
        - (2) Hungarian thread cultures
      - 3. influence of undesirable organisms
        - — "bloody," bitter, blue, ropy, slimy, and sweet-curdling milk
    - (b) commercial sour milks and starters
      - 1. higher per cent of acid (4 per cent in *B. bulgaricus*)
      - 2. form: tablets, liquid cultures
      - 3. wide range: efficiency, price; recent government comparison
      - 4. value of specially soured milks
        - (1) effect on bacterial flora of alimentary tract
          - — compare effect of ordinary milk
        - (2) effect on protein decomposition
          - — compare utilization of other protein food taken with milk
- (C) Butter
  - a. persistence of disease organisms
  - b. methods
    - (a) usual home methods — wide range in
      - 1. chance bacterial flora
      - 2. aroma
      - 3. undesirable flavors
      - 4. over-ripe results
    - (b) creamery methods
      - 1. pure cultures, or "starters"
      - 2. why milk pasteurized first
      - 3. western creamery processes (with soured milks)
    - (c) effect of salt on bacteria
      - — actual salt concentration high
- (D) Cheese
  - a. kinds depend on
    - (a) milk: whole milk, skim milk, goat milk
    - (b) additions made: cream, water, bread, sage
    - (c) organisms
      - 1. organisms present
      - 2. their sequence — most important
      - 3. starters
        - — methods of distributing: molds, gas-bacteria
    - (d) temperature

(e) time occupied in souring, curing, ripening

b. description of flora and method of making two cheeses

(a) American or cheddar cheese

(b) one other commercial cheese (see demonstration)

LABORATORY EXERCISES ON MILK

1. Effect of Pasteurization

a. Shake well the sample of milk provided and divide it into two parts, pouring each half into a sterile test tube. Pasteurize one tube (80° C. for 1 minute) and cool quickly by placing in the ice bath provided. Keep the non-pasteurized tube as a control.

b. Make 2 milk plates (1/100 dilution and 1/10 dilution) from each of the test tubes. Use either the ordinary pipette method or that described* at the foot of this page.

c. Incubate 2 days at 37° and then compare the results per c.c. for pasteurized and non-pasteurized milk.

2. Commercial Sour Milks

a. Fill two tubes ½ full of milk. Plug and pasteurize both, cooling quickly (ice bath).

b. Inoculate one with the commercial sour milk provided. Make a smear of the sour milk used, dry the slide and keep it for the next exercise. Leave both tubes at room temperature, examining them after 24 and 48 hours. What change in their gross appearance?

c. Will pasteurized milk sour? Even if not inoculated with lactic acid organisms?

d. Make smears of each of the pasteurized tubes (a and b above). Stain these and also the commercial milk slide made last time (see b). (Use xylol, alcohol, and then methylene blue, as in staining the cheese slide. What objection to fixing milk slides with heat?)

How many types of organisms in the commercial sample? In your tube inoculated from it? Explain the bacterial flora of the non-inoculated tube. What relation between spore production and the persisting types? Do the original forms show in the inoculated milk? Explain.

MILK DEMONSTRATION

Raw Milk

1. The Cell Content of Milk, Ross, (Bull. 303, Dept. Dairy Ind., Cornell University).

2. Microscope slide of raw milk (stained with methylene blue). In the center note white corpuscles (leucocytes) with two or more nuclei. See drawing also. Too many are considered a sign of inflammation and probably indicate diseased conditions in the udder or the near-by lymph nodes. Authorities differ about *the number allowed with safety in milk*. The round unstained areas in this slide are due to the oil globules present in the milk. Compare this slide with the one you have made for yourself, in which you removed the fat first. Why is that necessary for accurate microscopic work?

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*Mechanical devices for lessening the routine work are often used. For milk plates a small brass ring has been used in the N. Y. City Research Laboratory to secure a given volume of milk, 1/100 c.c. Such a ring (sterile, of course) is dropped into a milk sample, hooked out with a sterile wire hook, and dropped directly into the melted agar. What advantages with regard to the supply glassware? sterilized materials? time? training necessary for the workers? What disadvantages in this abbreviated method?

3. Another smear of raw milk. How many bacteria are visible? This field shows but a small portion of the 1/100 c.c. of milk which was spread on the slide, covering a space of two sq. cm. How can we determine the number in the whole 1/100 c.c.? In one c.c.? (See 2 below.)

Milk Examination

1. Special pipettes used to deliver small, known amounts of milk. Read manufacturer's description (Spencer) of apparatus for direct counts.

2. A New Method of Determining Milk Quality by Hall (Brew and Breed), Pop. Ed. of Bull. Nos. 373 and 380, 1914. Dead bacteria may take stains much as live bacteria do. Will the direct method be of value in examining pasteurized milk? Explain. Who would suffer if this test were accepted for pasteurized milk — the producer or the consumer?

3. Counting Bacteria by Means of the Microscope, Breed & Brew, (Tech. Bull. 49, N. Y. Agric. Sta., Geneva, N. Y.). Those interested in technique will find this more helpful.

4. Plate or count method of examining milk. Dilutions (1/10, 1/100, 1/1000) of a sample of milk were used to make these three plates. Are the relative numbers what you would expect? Explain the apparent effect upon the size of the colonies.

5. The sample used above was afterward pasteurized. Compare the plate made from it then with its corresponding unpasteurized dilution.

Pasteurization

1. Directions for the Home Pasteurization of Milk, Rogers (Circ. 152, Bureau An. Ind., 1909).

2. The Bacteriology of Commercially Pasteurized and Raw Market Milk, Ayers & Johnson, (Bull. 126, Bureau An. Ind., 1910).

3. The Pasteurization of Milk, Ayers, (Circ. 184, Bureau An. Ind., 1912).

4. Pasteurization of Milk (etc.), Kilbourne, (Reprint 1, Dept. of Health, New York City, 1915).

5. Pasteurizing Milk in Bottles (etc.), Ayers, (Bull. No. 240, Bureau An. Ind., 1915).

6. The Present Status of the Pasteurization of Milk, Ayers, (Bull. 342, Bureau An. Ind., 1916).

Production of Clean Milk; Care of Milk

1. The Production and Care of Milk, (Special Circular, Bureau An. Ind.).
2. Production of Clean Milk, (Farm. Bull. 602, Bureau An. Ind., 1914).
3. Bacteria in Milk, Rogers, (Farm. Bull. 348, Bureau An. Ind., 1909).
4. Ropiness in Milk & Cream, Ward, (Bull. 195, Ag. Exp. Station, Cornell University).
5. The Invasion of the Udder by Bacteria, Ward, (Bull. 178, as above).
6. The Control of Bulk Milk in Stores, Kelly, (Circ. 217, Bureau An. Ind., 1913).
7. The Application of Refrigeration to the Handling of Milk, Bowen, (Bull. 98, Bureau An. Industry).
8. Germ Content of Stable Air and Its Effect upon the Germ Content of Milk, Ruehle and Kulp, (Bull. 409, New York Ag. Exp. Station, Geneva, N. Y.).
9. Utensils, etc.
    a. Illustrations of preferred form of utensils for milking.
    b. A Simple Steam Sterilizer for Farm Dairy Utensils, Ayers and Taylor, (Farm. Bull. 748, 1916).
    c. Vacuum Cleaning as Applied to Dairy Cows, Ruehle, (New York Ag. Exp. Station, Geneva, N. Y.).
    d. The Milking Machine as a Factor in Dairying, Lane and Stocking, (Bull. 92, Bureau An. Ind., 1907).

10. Score card system. Examine the factors listed on the score card of the N. Y. College of Agriculture at Cornell. Would you *change* (raise or lower) any of these credits? Why?

It is estimated that moistening the udder with a damp cloth and using a small-topped pail will reduce the number of bacteria 90 per cent (in healthy cows, of course). Are these two factors valued high enough in this score card?

Disease organisms known to be carried by milk

1. Diphtheria. Note the unequal way these organisms stain. Compare with the illustrations. Why do physicians take a throat swab in order to diagnose this disease? Why is such direct microscopic examination more helpful in diagnosing this disease than in most human diseases?

2. Pseudo-diphtheria. Compare with the preceding; it differs mainly in lacking pathogenic qualities. Diphtheria is an organism which may develop involution forms. This fact, and the striking similarity of the pseudo-forms, make diagnosis more difficult than the previous microscope legend implied.

3. Human tuberculosis. Tuberculosis organisms (as earlier described) stain with difficulty (waxy envelope); once stained, they hold the stain even when bleached in strong acid (10 per cent nitric) or acid and alcohol, (3 per cent HCl in 95 per cent alcohol). They are therefore called acid-fast. Few, if any other, acid-fast organisms are found in sputum; finding such organisms is therefore usually considered conclusive. Note also the characteristic, finely-granular character of these very slender rods.

4. Bovine tuberculosis. Compare these in size and staining characters with the human type. In N. Y. City it is estimated that about 12 per cent of the cases in little children are due to the bovine type. Why should milk for babies be pasteurized?

5. *B. coli* and *B. typhosus* — slants only. The slides were shown in the water demonstration. Many epidemics of typhoid have been traced to milk. *B. coli* tests are used to test the possible fecal contamination and therefore undesirable condition of milk and ice cream.

6. Articles and pamphlets on several of the recent sorethroat epidemics due to streptococci; how many of them are traced to milk?

7. Other milk-borne diseases and epidemics are described in such journals as these: Public Health News (N. J.), Health News (N. Y.), government bulletins, and various medical journals. Read at least two.

Milk Regulations

1. Rules and Regulations Relating to the Sale of Milk and Cream, (Dept. of Health, New York City, 1914).

2. Regulations concerning Milk (extracts from the 1915 Sanitary Code of New York City).

3. A collection of labels and caps used for the various grades of milk sold in New York City.

4. Medical Milk Commissions and the Production of Certified Milk in the United States, Lane, (Bull. 104, Bureau An. Ind., 1908).

5. Note in this full-page advertisement from a daily paper how the milk companies respond to the demands of an alert public. This company promises (a) inspected cows; (b) stable cleanliness; and (c) iced milk cans. Would such an advertisement have been used ten years ago? Is the consumer fully protected by the conditions described in this advertisement?

Books on Milk and Milk Products. Look through these books; select one for your reference reading on milk and cheese.

1. Savage:	Milk and the Public Health (Macmillan)
2. MacNutt:	The Modern Milk Problem (Macmillan)
3. Rosenau:	The Milk Question (Houghton, Mifflin)
4. Klein:	Principles and Practice of Milk Hygiene (Lippincott)
5. Wing:	Milk and its Products (Macmillan)

6. Dairy Industry in New York State (N. Y. State Dept. of Agriculture)
7. Larsen & White: Dairy Technology (Wiley)
8. Van Slyke: Modern Methods of Testing Milk and Milk Products (Judd)
9. Meyer: Modern Butter Making (Meyer, Madison, Wis.)
10. McKay & Larsen: Principles and Practice of Butter making (Wiley)
11. See also Marshall's Microbiology, Lipman's Bacteria in Relation to Country life, and Conn's Agricultural Bacteriology (Blakiston)

Milk Products

1. Slide showing organisms found in a commercial buttermilk. Identify a yeast, a mold with large cylindrical cells, and bacteria. How does the flora of this culture compare with your own inoculated milk?

2. Fermented Milks, Rogers, (Bull. 319, Bureau An. Ind., 1916).

3. Circular advocating the various "Metchnikoff" milk products. How do the claims (marked passages) compare with the value given such milks in the lecture, the above pamphlet, or in the reference readings?

4. Slides showing several different cheeses
   a. Cheddar, American, or store cheese
   b. Limburger
   c. Cream
   d. Roquefort

Which contain molds? yeasts? What morphological varieties of bacteria in each? Do all the important bacteria show in a slide made from a finished cheese? Are the forms seen here all responsible for the present flavor and texture of these cheeses?

5. The Determination of Bacteria in Ice Cream, Ayers and Johnson, (Bull. 563, 1917).

6. The Pasteurization of Dairy By-Products, Dotterrer & Breed, (Bull. 412, N. Y. Ag. Exp. Station, Geneva, N. Y.).

7. Representative Pamphlets on Cheese-making.
   a. Fungi in Cheese Ripening, Thom, (Bull. 82, Bureau An. Ind., 1906).
   b. Defects in American Cheddar Cheese, Publow, (Bull. 257, Cornell Ag. Exper. Station, Dairy Dept., 1908).
   c. The Bacteriology of Cheddar Cheese, Hastings, Evans, and Hart, (Bull. 150, Bureau An. Ind., 1912).
   d. Bacteria concerned in the Production of Characteristic Flavor in Cheese of the Cheddar Type, Evans, Hastings, and Hart, (Journ. Agricultural Research, No. 3, 1914).
   e. Relation of the Action of Certain Bacteria to the Ripening of Cheese of the Cheddar Type, Hart, Hastings, Flint, and Evans, (Journ. Agricultural Research, No. 3, 1914).

XIII. ECONOMIC PHASES OF BACTERIOLOGY (NOT INCLUDING SOILS)

Illustrative material: table of soil changes (Fowler); charts with fermentative formulas (alcohol to acetic acid, etc.).

A. Removal or inhibition of micro-organisms
  (A) Canning
    a. early history; previous and contemporary discussion of "spontaneous generation"
    b. methods
      (a) ordinary home methods
      (b) discontinuous sterilization
      (c) increased temperatures: oil bath (temperature of boiling oils), added salts, steam pressure (why preferred in commercial processes)
  (B) Preserving (jellies, jams, etc.)
    a. better keeping qualities
      (a) less water — plasmolysis of organisms
      (b) absence of oxygen
        1. air expelled by long boiling
        2. molds, etc., usually limited to aerated part of jar
      (c) formaldehyde (prolonged heating of sugar)
  (C) Brines, pickles, etc.
    a. salt meat, fish, etc.
    b. bouillon cubes
    c. catsups — high bacterial content allowed by "pure food" laws
  (D) Removal of water
    a. dried meats — slight water content of outer part
    b. other animal products
      (a) milk — condensed; powdered
      (b) eggs
        1. powder for cooking purposes
        2. compare deterioration when kept in shell
    c. fruits, vegetables
      (a) primitive methods
      (b) now dried quickly to prevent color changes
        1. artificial heat
        2. pressure to remove water
    d. hay, etc. (fermented instead, in localities lacking favorable climatic conditions)
  (E) Low temperatures
    a. range: home icebox to refrigerating plants and cars; fruits to whole animals; foods to fur coats
    b. advantages
      (a) control of micro-organisms
      (b) conservation and distribution of seasonal and temporary products
      (c) meats and fish
        1. results of chemical tests
        2. effect on trichina
        3. advantage gained through U. S. inspection required on interstate exchanges (affecting major part of cold storage meats and fish)
    c. attending evils
      (a) deterioration during storage
        1. cell enzymes in original substances themselves (canned fruit)
        2. bacteria

- (1) chickens drawn and undrawn
- (2) bacterial content of eggs
- (b) rapid changes following cold storage caused by
  - 1. water of condensation
  - 2. improper methods of chilling and thawing
    - (1) ice in contact with meat, etc.
    - (2) water baths (water often soiled)
    - (3) repeated thawings (why forbidden?)
  - 3. prolonged high temperature after removal from storage
- (F) Addition of chemicals
  - a. action of — discussed earlier
  - b. chemicals commonly used
  - c. objections to
    - (a) effect on consumers
      - 1. directly injurious
      - 2. possible cumulative effect
      - 3. may make foods less digestible (formaldehyde fixes proteins)
    - (b) used to mask poor materials — effect on color, etc.
  - d. government regulations
    - (a) wisdom of complete labels
    - (b) labels alone insufficient protection
      - 1. ignorant purchaser
      - 2. unsuspecting consumer
- (G) Silo (see p. 71 also)
  - a. limited growth of bacteria due to
    - (a) heat
      - 1. oxidation — plant cells (cell enzyme changes)
      - 2. bacterial action
    - (b) limited amount of oxygen
    - (c) accumulation of ferment products
- (H) Soil (See also Soils and the Nitrogen Cycle)
  - a. Rothamstead theory — heating to remove undesirable micro-organisms
  - b. relation between heated soils and per cent of soluble salts and organic acids (Clark and Seaver)
  - c. steam harrow (Stone) for treating soils

B. Controlled growth of micro-organisms
(Bread, milk products, and soils treated elsewhere)

- (A) Soured and pickled foods of indefinite bacterial flora: sour krout, catsups
- (B) Wines — "product of alcoholic fermentation of grapes plus usual cellar treatment"
  - a. numerous varieties; main classes: dry, sweet, fortified, sparkling, unfermented
  - b. starters
    - (a) pure cultures of yeast; gaining in use
    - (b) handful of soundest grapes
    - (c) vineyard soil
  - c. yeasts
    - (a) present in vineyard soil
    - (b) related to: dust, moist surface of grapes, breaks in grape skins, presence of bees and wasps
    - (c) varieties
      - 1. several in any district
      - 2. more diverse than beer yeasts
      - 3. alcohol may reach 16 per cent

- 4. undesirable kinds
  - (1) how detected
  - (2) effects of
- d. molds always present
  - (a) common types
    - 1. related to broken surfaces, etc., as yeast
  - (b) effects
    - 1. may use up sugar and alcohol
    - 2. destroy flavor or "brightness"
    - 3. cause objectionable odors and tastes
- e. bacteria
  - (a) common — surface of vats
  - (b) injurious effects
- f. physical phases of wine making
  - — processes: fining, filtering, precipitating, decanting
  - — albuminous clearing agents: isinglass, white of egg, gelatine

(C) Beer
- a. changes due to
  - (a) enzymes of grains
  - (b) of yeasts
    - 1. addition of yeasts, 8-18 days
    - 2. top and bottom yeasts and resulting types of beers
- b. stages in manufacture
  - (a) fermentation
    - 1. starch to sugar
    - 2. fermentation of sugar
  - (b) coagulation of proteins and clarifying
    - 1. wood chips
    - 2. also as in wines
  - (c) hopping, etc.
    - 1. preserve aroma
    - 2. antiseptic properties
  - (d) pasteurization and bottling or casking
- c. diseases
  - (a) related to low acid content
  - (b) types of

(D) Distilled liquors
- a. made from starchy or sugary materials
- b. agents
  - (a) molds often useful in first stages
  - (b) distillery yeasts yield higher per cent of alcohol
- c. kinds
  - (a) potable
    - 1. brandy (grapes)
    - 2. rum (sugar cane)
    - 3. whiskey (grains)
  - (b) industrial
    - 1. any substance that will ferment
    - 2. cost main consideration
    - 3. examples

(E) Vinegar
- a. changes
  - (a) mainly bacterial
  - (b) alcohol (from yeasts) to acetic acid
    - — power possessed by many organisms
- b. substances — any material yielding alcohol: potato, molasses, fruits

- c. results depend on
    - (a) amount of alcohol
        - 1. inhibits bacteria
        - 2. inhibits yeasts themselves
    - (b) resistance of acetic organisms
    - (c) access of oxygen
- d. methods differ mainly in modifications expediting contact of bacterial film and liquid with optimum amount of oxygen
- e. vinegar eels
    - — injurious effects
    - — how removed

(F) Commercial processes where little is known of the micro-organisms causing the desired changes

- a. tanning of leather
    - (a) advantages of preliminary salting, drying
    - (b) more or less controlled decomposition of leather
        - 1. substances used to cause such changes
        - 2. results
- b. preparation of fibers (flax, hemp, etc.)
    - (a) structure of plant stems and leaves
    - (b) process possible through difference in rate of decay of fibers and parenchyma cells
    - (c) describe old hand-method of rotting, retting, etc.
    - (d) recent bacterial aids — pure cultures to remove color (pectin) from cell walls

(G) Processes depending markedly on changes due to enzymes in cells of plants concerned (and probably *less* upon bacterial enzymes)

- a. methods — adapted to securing
    - (a) optimum temperatures, e.g., "sweating"
    - (b) optimum oxygen supply
- b. plants, etc., used
    - (a) indigo
        - 1. sources — plants (indican); (also coal tar)
        - 2. cell enzymes important
            - (1) shown by factors affecting product (time of day picked, etc.)
    - (b) tobacco
        - 1. method of drying, ripening
        - 2. changes produced
            - (1) loss nicotine
            - (2) aroma and color
                - — advantages of slow drying
        - 3. relation of locality to product
    - (c) tea
        - 1. quality depends on relation of oils, unfermented tannin, and "body"
        - 2. bacteria really necessary?
        - 3. important considerations
            - (1) rate of drying
            - (2) heat
            - (3) antiseptics
    - (d) cocoa
        - 1. partial fermentation
            - (1) chemical effects: taste; color
            - (2) physical gains: loosens seed coat, and also pulp from seed
    - (e) coffee (changes much as in cocoa)
    - (f) meats
        - 1. cell enzymes and *rigor mortis*
        - 2. "high" or "gamy" flavors due to bacterial changes

- (g) silo
  - — discussed earlier under inhibition of bacterial action
  - — practically nothing known of organisms concerned; *B. bulgaricus* important?
- (H) Cellulose — enzymes decomposing cellulose important
  - a. plant decay
  - b. digestion of foods
    - (a) cellulose in plant cell walls
    - (b) food liberated
      - 1. breaking cell wall
        - (1) cooking
        - (2) crushing
        - (3) chemical action on cellulose
          - — enzymes (e.g., intestinal)
          - — bacterial enzymes
      - 2. cellulose itself of food value
    - (c) bacteria digesting cellulose
      - 1. common in soil
      - 2. herbivorous animals
- (I) Sewage problems
  - a. organic material in sewage
  - b. decomposition of organic material
    - (a) phases
      - 1. anaerobic bacteria — septic tank
      - 2. aerobic bacteria — contact beds or activated sludge tanks
        - (1) rapid denitrifying processes
        - (2) action increased by increasing exposed surface: sprays, trickle beds, etc.
      - 3. filter — used
        - (1) to clear water — for manufacturing purposes
        - (2) to separate sludge used as fertilizer
        - (3) often combined with contact beds
      - 4. disinfection
        - — of part retained for use
        - — high quality obtainable
    - (b) description of one plant for sewage disposal

DEMONSTRATION — ECONOMIC PHASES OF BACTERIOLOGY

Plant Diseases

Most important economically are the diseases that affect our staple crops. Most of these are due to fungi; bacteria, however, are responsible for a large share of the yearly losses.

1. Diseases of Economic Plants, Stevens and Hall, (Macmillan).

Look through to get a general idea of the plants affected by diseases due to fungi, bacteria, etc.

2. Fungous Diseases of Plants, Duggar, (Ginn).

3. Bacteria in Relation to Plant Diseases, Erwin F. Smith, (Carnegie Institution, Washington).

A most comprehensive publication on plant diseases. Vol. I is devoted mainly to technique. Read here and there to see if it differs greatly from ordinary bacteriological technique. Vol. II describes the various plant diseases. Look at the illustrations and their legends. Read the description of the plant disease that interests you most. How is the disease transmitted?

4. The following pamphlets on bacterial diseases illustrate the work being done by the U. S. Government, the state experiment stations and agricultural colleges:

a. A Bacterial Disease of Tomato, Eggplant, and Irish Potato, Smith, (Bull. 12, U. S. Dept. Ag., 1896).
b. A Bacterial Stem Blight of Field and Garden Peas, Sackett, (Bull. 218, Colo. Ag. Col., 1916).
c. Hibernation of Phytophthora Infestans of the Irish Potato, Melhus, (Journ. Ag. Research, Vol. 5, p. 71, 1915).
d. Peach Leaf Curl, Pierce, (Bull. 20, Dept. Ag. Dept. Veg. Phys. & Path., 1900).

Animal Diseases

These cause great losses to our country every year. Their importance may be judged by the following marked publications.

1. a. Tuberculosis in Cattle and Its Control, Law, (Bull. 150, Ag. Exp. Station, Veterinary Div., Cornell University).
b. Tuberculosis of the Food-Producing Animals, Salmon, (Bull. 58, Bureau An. Ind., 1906).
c. Bovine Tuberculosis and Its Control, Moore, (Carpenter, Ithaca, N. Y., 1914).

2. Actinomycosis or Lumpy Jaw, Salmon, (Circ. 7, Bureau An. Ind.).

3. Bacteria of the Apiary, White, (Tech. Ser. 14, U. S. Bureau of Entomology, 1906).

4. Rabies and Its Increasing Prevalence, Hart, (Circ. 129, Bureau An. Ind., 1908).

5. Texas Fever, Mohler, (Bull. 78, Bureau An. Ind., 1905).

6. Protozoan Parasites of Domesticated Animals, Crawley, (Circ. 194, Bureau An. Ind.).

7. Further Studies with Reference to Spirochaetes Observed in Swine, King, Drake & Hoffman, (Reprint 71, Parke, Davis & Co., 1914).

8. Foot and Mouth Disease, Mohler, (Farm. Bull. 666, 1915).

Meat and Other Foods

1. Regulations Governing the Meat Inspection of the U. S. Dept. of Agriculture, 1914.

It is the *Interstate* character of the meat industry that enables the U. S. Government to rule concerning the slaughter of animals, and the sale of meat and meat products. What are the rules concerning the slaughter and sale in your own state? A large number of small low-grade slaughter houses are said to flourish near all great packing house centers; does the fact that they sell only within the state where they are located throw any light upon the comparative value of *state* and *interstate* regulations?

2. Food Regulations of the Department of Health, (New York City, Reprint 31, 1915). See chapter "Contents" and marked passages. Does your experience in this city make any additions necessary? Are these printed rulings enforced?

3. Vinegar: a. Vinegar containing vinegar eels. Hold to the light; use a hand lens if necessary to see the wriggling eels.

b. Smear made of "mother of vinegar," showing the bacteria composing the "mother." Most of these are probably the vinegar organisms which oxidize alcohol to acetic acid. C_2H_5OH (alcohol $+ O_2$) $= C_2H_4O_2$ (acetic acid) $+ H_2O$.

4. Representative pamphlets on food preservation.

a. Handling of Dressed Poultry, Pennington, (Dept. of Agriculture Year book, 1912).
b. The Comparative Rate of Decomposition in Drawn and Undrawn Poultry, Pennington, (Circ. No. 70, Bureau of Chemistry, U. S. Dept. of Agriculture).
c. Bacteriology of Eggs, Rettger, (Centrl. Bakt., 39, p. 611, 1913).

d. Bacteriological Study of Shell, Frozen, and Desiccated Eggs, Stiles and Bates, (Bull. 158, Bureau of Chemistry, U. S. Dept. of Agriculture).
e. The Production and Handling of Market Eggs, (Bull. No. 5, Bureau of Chemistry, U. S. Dept. of Agriculture).
f. The Bacterial Infection of Fresh Eggs, Hadley and Caldwell, (Bull. 164, R. I. Ag. Exp. Station, 1916).
g. Shellfish Contamination from Sewage-polluted Waters and from Other Sources, Stiles, (Bull. 136, Bureau Chem., 1911).
h. Decomposition and Its Microscopical Detection in Some Food Products, Howard, (Bureau Chem., Dept. Ag. Year book, 1911).
i. A Successful Community Drying Plant, Pugsley, (Farm. Bull. 916, 1917).
j. Drying Fruits and Vegetables in the Home, (Farm. Bull. 341, 1917).
k. Commercial Evaporation and Drying of Fruits, (Farm. Bull. 903, 1917).
l. Home Canning by the One-Period Cold-Pack Method, Benson, (Farm. Bull. 839, 1917).
m. Successful Canning and Preserving, Powell, (Lippincott, 1917).
n. Preservation of Vegetables by Fermentation and Salting, Round and Lang, (Farm. Bull. 881, 1917).

XIV. SOILS AND THE NITROGEN CYCLE

Illustrative material: Pea plants with nodules; photographs of beans grown with and without nodule bacteria; charts contrasting denitrification, and nitrogen-fixation; diagram of nitrogen cycle.

A. Soils
    (A) Water problems
        a. direct control
            — irrigation
            — drainage
        b. indirect control
            (a) change mechanical condition of soil (e.g., effects of lime)
            (b) forest relations
    (B) Minerals
        a. kinds present important
            — as food
            — as stimulants to growth
        b. relation to prospective crops
        c. strength of soluble mineral salts important (plasmolysis)
        d. natural plant decay, and return of nutritive elements to soil
    (C) The nitrogen problem
        a. protein character and needs of all cells
        b. soil nitrates constantly need replacing
            — compare other minerals in amount and form returned to soil
        c. forms in which supplied to plants
            (a) commercial fertilizers
            (b) bacterial deposits
            (c) fixation of atmospheric nitrogen
                1. by flame (hydrogen gas)
                2. electricity
                3. ultra-violet light
                    — returned to earth by rain, snow
                4. combination with dextrose and cellulose (rotting wood)

- (D) Efforts to improve soils
  - a. allowing grounds to lie fallow
    - (a) effect upon
      - 1. physical condition
      - 2. minerals
      - 3. molds, bacteria, insect pests
    - (b) effects not always beneficial; reasons
  - b. heating soils
    - (a) effect on mold and insect pests
    - (b) effect on micro-organisms
    - (c) effect on organic acid and salt content
  - c. inoculation of soil with beneficial organisms (discussed below)
- (E) Three main bacterial phases of the soil problem
  - a. denitrification
    - (a) stages (chart)
    - (b) term applied loosely to whole or part of denitrifying process
    - (c) organisms — numerous and wide spread
    - (d) temperature important
    - (e) prerequisites or conditions
      - — limited oxygen and carbohydrates
      - — nitrogenous material
    - (f) process interfered with by upbuilding of nitrates into insoluble proteins
    - (g) stages during process favorable to general plant growth
      - 1. absorbable peptones, etc., (slight)
      - 2. nitrates
        - — readily decomposed to N, NH_3, and so lost
        - — "leaching" of manure
  - b. nitrogen-fixation (by molds also)
    - (a) soil bacteria
      - 1. mainly aerobic
      - 2. considerable resistance to drying, etc.
      - 3. most common species
        - — characters
        - — soil depths
      - 4. necessary elements
      - 5. nitrates deposited in soil
        - — amount per acre; Colorado deposits
      - 6. commercial forms
    - (b) nodule or root bacteria
      - 1. plants bearing nodules
        - — symbiosis claimed
        - — benefits even crop of present season
      - 2. history of study of root nodules
        - — old ideas concerning nodules
      - 3. related to host plant
        - — species
        - — resistance of root hairs
      - 4. results affected by
        - — carbohydrates present
        - — minerals
      - 5. amount of nitrates formed
      - 6. rotation of crops
        - — nitrates deposited by one crop of series
        - — mechanical advantages: texture, depth of cultivation, etc.

            — common rotation series
        7. commercial forms
            — methods of preparing
            — methods of inoculating soils
- c. nitrification
    - (a) historical development
        1. suggested by Pasteur
        2. experiments proving nitrification
    - (b) two phases of process performed by different organisms
        1. ammonia to nitrites
        2. nitrites to nitrates
    - (c) affected by
        1. temperature
        2. minerals present
        3. oxygen supply
    - (d) common species changing
        1. ammonia to nitrites
            — *cycle* in development of organisms
            — species local in distribution
            — affected by organic substances
            — CO_2 utilized
        2. nitrites to nitrates
            — names
            — ammonia injurious
            — organic matter less inhibitory

- (F) Mineral cycles related to plant needs
    - a. nitrogen cycle
        - (a) review on chart the part of the cycle due to bacteria
            — cross cuts and "leaks" in the cycle
        - (b) interrelation of green plants and bacteria
    - b. other cycles, e.g., carbon cycle, sulphur cycle, etc.
        - (a) green plants and micro-organisms not necessarily strict alternates, e.g., sulphur cycle
        - (b) cyclic changes not necessarily limited to biological relationships or combinations, e.g., oxygen or iron cycles

SOIL DEMONSTRATION

1. A sample of wood earth was incubated 18 hours in broth. How many kinds of organisms show in this drop taken from that broth? Does this necessarily show all the forms present in that soil? Does this slide tell you anything of the number (per c.c.) actually present in that soil?

2. *B. radicicola* — stained with carbol-fuchsin. Compare with the illustrations. This species varies greatly in shape.

3. Pea roots, showing nodules, filled with bacteria, *B. radicicola*. One of these nodules was sectioned to make the following slide.

4. In this section of pea roots find granular-looking cells, containing *B. radicicola;* see drawing.

5. Photograph showing peas grown (soil) with and without nitrate-forming bacteria. Explain.

6. Photograph showing beans grown (water solutions) with and without nitrates. Explain.

7. Representative pamphlets on soil and legume bacteria.
    - a. Culture Media for Use in the Plate Method of Counting Soil Bacteria, Conn, (Tech. Bull. 38, N. Y. Ag. Exp. Station, Geneva, 1914).
    - b. Bio-chemical Studies of Soils Subjected to Dry Heat, Seaver & Clark, (Contrib. 154, New York Bot. Gard., 1902).

- c. Bacteria and the Nitrogen Problem, Moore, (Reprint from 1902 U. S. Yearbook).
- d. Legume Inoculation, Prucha, (Circ. 15, New York College of Agriculture, Cornell University, 1913).
- e. Conditions Affecting Legume Inoculation, Kellerman & Robinson, (Bull. 100, Part 8, Bureau Plt. Ind., 1906).
- f. Farmers' Bulletins, such as these: Nos. 455, 550, 579, and 924.

XV. HEALTH AND DISEASE

Illustrative material: Charts showing diseases traced to bacteria; to protozoa; list of diseases for which causal organisms have not been ascertained.

- A. Pathogenicity of organism dependent upon
    - (A) Toxins
    - (B) Ability to grow and multiply in body
        - a. power of using host proteins
        - b. power of resisting activities of host cells
    - (C) Poisons of "split proteins" (Vaughn)
- B. Modes of entrance
    - (A) Limited by natural body defences
        - a. skin itself
        - b. germicidal and antiseptic secretions; tears, nasal secretions, bile, etc.
        - c. unfavorable conditions in body
            - (a) available food supply
            - (b) lack of oxygen in tissues themselves
            - (c) germicidal qualities of blood
    - (B) Mainly through mouth and nose
        - a. foods
        - b. hands; value of frequent washing of hands
    - (C) Through skin
        - a. entrance unbroken skin uncertain — except protozoan diseases
        - b. entrance through breaks in skin
        - c. compare bacteria normally present on surface of body
    - (D) Preferred methods of entrance of many bacteria
        - a. subcutaneous, (e.g., tetanus)
        - b. alimentary canal (e.g., typhoid)
        - c. nasal and respiratory regions (e.g., pneumonia)
- C. Modes of exit
    - (A) Mouth and nose
        - a. death rate of bacteria in air; effect of mucous covering
    - (B) Urine and feces
        - a. so-called "filth diseases"
        - b. danger from carriers, e.g., cooks, waiters, and dairymen —history of several known carriers and epidemics
- D. Diseases named
    - (A) From causal organism
    - (B) Locality affected
        - a. given disease due to various organisms
        - b. given organism causing various diseases
- E. Reactions of the body to disease
    - (A) Increase in the number of white corpuscles
        - a. action of leucocytes on bacteria

- b. methods of measuring
- (B) Formation of definite antisubstances (discussed later in detail)
  - a. against bacteria; or any foreign cells
  - b. probably formed mainly by lymph and "marrow" cells
  - c. nature of such antibodies
    - (a) neutralize bacterial action
    - (b) destroy bacteria
    - (c) modify bacterial action and resistance
- (C) Fever
  - a. as a measure of cell activity, not the disease
  - b. induced fevers (Vaughn)
  - c. effect of high temperatures on the body cells

F. Antibodies formed in response to invading organisms
- (A) Antitoxin
  - a. cell receptor theory
    - (a) foods and cells
    - (b) toxins and cells
    - (c) antitoxins as thrust-off receptors
    - (d) phenomena supporting theory
      - — neutralization of toxin
      - — malnutrition and predisposition to disease
      - — results of combined beef broth and toxin injections
  - b. production of diphtheria antitoxin
    - (a) preparation of toxin
    - (b) horse phase
      - 1. inoculation with toxin
      - 2. collection of blood
        - (1) period
        - (2) amounts
    - (c) purification of collected serum
      - 1. laboratory procedures
    - (d) strength of the antitoxin
      - 1. method of testing
      - 2. dosage
  - c. results of antitoxin treatment
    - (a) passive immunity (active in horse)
    - (b) compare results when whole serum used
    - (c) anaphylaxis (see below)
- (B) Other antibodies affecting bacteria or foreign cells directly
  - a. lysins: bacteriolysins; haemolysins; cytolysins
    - (a) action on invading organisms more direct than in other antibodies
    - (b) applications
      - 1. blood and meat tests
      - 2. diagnosis of disease
      - 3. transfusion of blood
  - b. aids to white corpuscles
    - (a) agglutinins and precipitins
      - 1. effect on invading organisms
      - 2. as tests for presence of disease
      - 3. as tests differentiating between cells, e.g., blood corpuscles of various species
        - (1) identification of meats
        - (2) murder investigations
    - (b) opsonins
      - 1. aiding bactericidal action of white corpuscles
      - 2. how measured
      - 3. opsonic index as measure of condition of subject

G. Immunity
  - (A) Active
    - a. ordinary recovery from disease
    - b. in diseases caused by
      - (a) similar organisms (smallpox and cowpox)
      - (b) attenuated organisms (rabies)
      - (c) killed organisms (tphoid)
  - (B) Passive
    - a. through whole serum or antitoxins formed by another animal
    - b. through drugs
      - (a) compare bacterial and protozoan susceptibility to chemicals
  - (C) Length of period of immunity
    - — elimination of antibodies
  - (D) Immunity and tolerance

H. Interesting applications or details of described body reactions
  - (A) Vaccine (bacterin) for typhoid; present and early definition of "vaccine"
  - (B) Treatment of rabies
  - (C) Tuberculin tests
  - (D) "Protein sensitives"
  - (E) Anaphylaxis

DEMONSTRATION: BACTERIA IN THE ANIMAL BODY, AND ITS REACTIONS TO THEM

1. Different types of *white corpuscles,* colored to show how different they are in (1) their size, number of nuclei, and granular character; and (2) in their selective action when stained with a mixed stain (such as methylene blue and eosin).

2. Blood (horse) showing a background of red corpuscles. This shows also a few white corpuscles. One is a highly differentiated form, an eosinophile, (picking eosin out of the mixed stain). Focus to see its very granular character — as if composed of many definite and similar units.

3. Red blood corpuscles showing various abnormal shapes characteristic of *anemia.* Normally the red corpuscles are coin-shaped as shown in 2.

4. How many kinds of white corpuscles are shown in this slide of normal human blood? How many poly-nuclear leucocytes are present?

5. A smear made from an inflamed surface, an infected eye. Note that the smear is composed almost entirely of white corpuscles. Can you explain their abundance here?

6. Serum from inflamed peritoneal cavity in a case of peritonitis. This serum has not been incubated; fresh material was smeared upon this slide and stained. The peritoneal vacity and all tissues are normally *sterile.* Note the heavy infection as shown by the numerous cocci (probably streptococci) and the masses of white corpuscles.

7. White corpuscles from an infected eye. Find one which has ingested *bacteria,* as in the accompanying photograph.

8. This slide shows the exudate from the nose in a grip-like cold sent in to a hospital for diagnosis; the specimen was not incubated, so the slide indicates the actual condition of the fresh exudate. Why should milkers wash their hands before milking? Is sneezing dangerous to one's companions? Give another important application or hygienic rule suggested by this exhibit.

9. The paler part to the right of this slide (ovary of a dog) is an abscess. Note that the cells are disintegrated in this region. To the left are normal cells, which are a little denser and much more definite. In some diseases, as tuberculosis, a capsule may be formed surrounding and isolating this disintegrated area, and the patient may entirely recover.

10. a. Pieces of tuberculous tissue from a cow. Note the large yellowish calcified area near (a) and the numerous small ones near (b).
    b. Look at the illustrations at the back of this book (Moore, Bovine Tuberculosis) and note the organs that may be affected in tuberculosis. Do these suggest any arguments for pasteurization of milk from all cattle which are not tuberculin-tested?

11. A little book which gives a clear exposition of the antibodies formed against bacteria, and explains their applications in diagnosis, prevention, and treatment of disease. Bolduan: Immune Sera (Wiley, 1911).

12. A brief review of the antisubstances formed by the body, and of the substances used to prevent and cure disease. Ferry: Infection and Immunity (Reprint 59, 1914, Parke, Davis & Co.).

13. A much more advanced book on infection than the preceding books. Each chapter or subject is so written that it is complete in itself. Zinsser: Infection and Resistance (Macmillan, 1914).

14. Various representative pamphlets on different phases of immunity, treatment of disease, e.g.,
    a. Biological Products (Veterinary) Wilson, (Reprint No. 29, 1912, Parke, Davis & Co.).
    b. The Treatment of Tetanus, (Reprint 51, 1913, Parke, Davis & Co.).
    c. Duration of Immunity following Smallpox Vaccination, (Reprint 48, 1913, Parke, Davis & Co.).

15. Syringe used to inject antitoxin, vaccine, etc. See label for dosage, etc.

16. Booklets by Mulford, and by Parke, Davis & Co., who manufacture antitoxins and other materials used in preventing and in treating disease. See Biological Products, Mulford Co. (p. 7-34); diphtheria antitoxin is discussed in a very readable way.

17. Packets like these are prepared by various Boards of Health for examination of suspects.

These (Boston Board of Health) are for the diagnosis of typhoid, malaria, and eye infections. See directions on each. The collected material or slides may be sent to the Board of Health for examination; many cities do this without charge. Why is this a legitimate way to spend the city revenues?

HEALTH AND DISEASE (WITH EMPHASIS ON THE TRANSFER OF DISEASE)

This exhibit includes a collection of more or less "popular" material, designed to awaken the public to the dangers attending the several phases of community life and to help establish practices less favorable to the spread of disease. Many of the pamphlets and articles are listed below. Others, received too late to be included in this list, are on the tables also. Look at the titles and marked passages. Find some which (1) will support you in your efforts to change certain procedures in your own home; (2) which will be useful in dealing with children; and (3) which will make an appeal to the most careless servant or ignorant parent.

Flies* Check the pamphlet most useful to you among the following:

1. A Minnesota Fly-trap, (Minnesota State Circular, No. 26, 1913).
2. A Campaign Against Flies, (Nature Study Review, Jan., 1911).
3. The House Fly and Disease, (Health News, July, 1915).
4. The Typhoid or Filth Fly, Hodge, (Nature Study Review, Oct., 1910).
5. The Filthy Fly, (New York State Department of Health).
6. Control of Flies as a Nature Study Problem; Hodge, (Nature Study Review, Feb., 1916).

Transfer of Disease

1. Death in Public School Drinking Cups, Davison, (Technical World Magazine, August, 1908, or Mass. Board of Health).

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*The headings in black type overlap more or less; e.g., the New York City code includes rulings belonging under each of these general headings.

2. The Bacterial Population of a Towel, Rosenberger, (Forecast Magazine, April, 1915).

3. The Relation of Lavatory Appliances to the Spread of Intestinal Infections, Rickards & Clore, (Science, p. 807, 1914).

4. Dogs and Disease, (Public Health News, April, 1916).

5. Lower Animals and Human Disease, (Public Health News, Oct., 1916).

6. Droplet Infection, etc., (Public Health News, Nov., 1916).

7. Quarantine Control of Contacts, (Health News, Nov., 1916).

8. New York State also issues each week timely articles (Health Hints) for publication in the various newspapers. The accompanying set issued during the past year includes such topics as the following:

   (a) The Deadly Drinking Cup;
   (b) Getting Ahead of Measles;
   (c) Flies or Babies;
   (d) Control of Whooping Cough;
   (e) Getting Rid of Mosquitoes;
   (f) Avoid Vacation Typhoid;
   (g) Typhoid Fever at a Church Dinner;
   (h) Dust and Disease.

Find some that will be of use to you in your future work.

Infant Welfare

1. Summer Care of Sick Babies, (Dept. of Health, New York City).

2. Infant Feeding Number, (Health News, May, 1914).

3. Infant Welfare Number, (Health News, June, 1915).

4. Baby Health Number, (Public Health News, March, 1916).

5. Directions for the Prevention of Ophthalmia Neonatorum (New York State Department of Health, Albany).

6. His Lordship, the Baby, (No. 10, Natl. Com. Prevention of Blindness, N. Y. City).

Common Diseases (especially among children)

1. Communicable Disease Numbers, (Health News; August, also September, 1914). See the chart in the August number; this gives rules for isolation, exclusion from school, etc.

2. School Principal's Number, (Health News, March, 1914).

3. Smallpox and Vaccination, (Health News, Feb., 1914).

4. Public Health Laboratory Number, (Health News, Dec., 1914).

5. The Rural School and Hookworm Disease, (Ferrell, Bull. 20, U. S. Bureau of Education, 1914).

6. Trachoma, (Its Prevalence, Effects upon Vision, Methods of Control), (National Committee for Prevention of Blindness, No. 6, 1915).

7. Whooping Cough, (Health News, March, 1917).

8. Tuberculosis Number, (Health News, Dec., 1915).

Special Diseases: Tuberculosis

1. Articles on Human Tuberculosis (including infection of man through bovine tuberculosis, milk, etc.; see also the sets of references on milk and animal diseases).

   a. Consumption, a Preventable and Curable Disease, (New York State Department of Health).
   b. Important Facts about Consumption, (New York State Department of Health).
   c. Relation of Bovine Tuberculosis to the Public Health, Salmon, (Bull. 33, Bureau An. Ind., 1901).
   d. Bovine Tuberculosis and Its Control, Moore, (Carpenter & Co., Ithaca, 1913).
   e. Milk and its Products as Carriers of Tuberculosis Infection, Schroeder, (Circ. 143, Bureau An. Ind., 1909).
   f. Tubercle Bacilli in Butter, Schroeder & Colton, (Circ. 127, Bureau An. Ind., 1908).
   g. Prevention of Tuberculosis, Newsholme, (Methuen, London).

2. Typhoid

a. Public Health Hints (N. Y. State Dept. Health) includes the following:
    Victims of a Typhoid Well
    Avoid Vacation Typhoid
    Typhoid Fever at a Church Dinner

b. Does the accompanying newspaper account of Typhoid Mary justify a broad interpretation of the powers of the Board of Health, Sanitary Code, 1915, sections 97 and 146?

c. This report by Houston (Sixth Research Report to the Met. Water Board, London, 1910) contains several interesting accounts of typhoid carriers.

d. Typhoid Fever, Whipple, (Wiley, 1908).

City and State Regulations

1. The Reporting of Disease, Dublin, (Reprint 202, U. S. Public Health Service).

2. For New York City and New York State the following rules and regulations have been framed. Glance at the marked passages to see the extent of the rulings thought necessary. What has your experience in New York City shown to be still needed? Consult these manuals to see whether it is due to a lack in the regulations, or the failure to enforce existing regulations.
    a. Public Health Manual, New York State Dept. of Health, 1914.
    b. Sanitary Code of the Board of Health, New York City, 1915.
    c. Rules and Regulations Governing the Practice of Midwifery in the City of New York, Board of Health, 1914.

3. The work of the New York State Department of Health for the last year is described in this issue of Health News. How does it compare with the work done in your own state?

Publications

1. Pamphlets issued free of charge to residents of New York State, New Jersey, or New York City. Does your state do anything of the kind?

2. Representative publications of special organizations: scientific, philanthropic and charitable have been shown from time to time. Make sure you have seen the following:
    a. Journal of Abstracts. A review of all the articles on bacteriology in most of the civilized world.
    b. The News Letter, (Nat'l. Com. for Prevention of Blindness, N. Y. City).
    c. Social Hygiene, (Am. Social Hygiene Assn.).

XVI. SOCIAL DISEASES

GONORRHOEA AND SYPHILIS

A. Reasons for including this subject in this course
- (A) Too general lack of correct information
- (B) False ideas of modesty or propriety
- (C) Diseases often contracted innocently and accidentally
    - a. knowledge necessary for self-protection
    - b. knowledge necessary (mothers and teachers) to safeguard children
- (D) Serious effects of these diseases
    - a. not limited to individuals affected (future generations)
    - b. effects
        - (a) general health
        - (b) sight
        - (c) sanity
        - (d) moral and mental control

- (E) Undue apprehension of many regarding accidental infection — organisms short-lived outside the body

B. Facts that should be known
  - (A) Methods of transfer
    - a. mainly sexual
    - b. often accidental (towels, drinking cups, etc.)
  - (B) Diseases not necessarily stigma upon individual

C. Gonorrhoea
  - (A) Causal organism
  - (B) How transferred
  - (C) Probable extent of infection in civilized countries
  - (D) Characteristics of the disease
  - (E) Effects — estimated by
    - (a) examination of large numbers of individuals
    - (b) operative records
    - (c) death rate
    - (d) blindness records
  - (F) Control of gonorrhoea
    - a. present status of preventive and curative efforts
    - b. present legal rulings
      - (a) reporting of disease
      - (b) methods employed by doctors and nurses

D. Syphilis
  - (A) Causal organism
    - a. characteristics
    - b. explanation of late discovery
    - c. cultivation methods
  - (B) Characteristics of the disease
  - (C) Methods of transfer
  - (D) Results serious — high percentages
    - a. infant mortality
    - b. nervous diseases and disorders
    - c. insanity and mental deficiency
  - (E) Curative therapy
    - a. present status
    - b. history of chemical treatment
    - c. permanency of cure
  - (F) Tests for syphilis
    - a. explanation of original Wasserman test
    - b. enzyme tests

E. Moral aspects of preventive and curative treatment

F. Civic, state, and U. S. efforts to abate these diseases

G. Personal applications
  - (A) Avoid over-emphasis in own mind
  - (B) Sanitary observances concerning
    - a. public and bathroom conveniences
    - b. towels, bedding, etc.
    - c. swimming pools
    - d. care of the eyes
    - e. frequent washing of hands
  - (C) Desirable attitude of women regarding
    - a. double standards of sexual morality
    - b. marriage
    - c. dress, social conventions, etc.

DEMONSTRATION FOR SOCIAL DISEASES

1. Organism causing syphilis; see illustration.

2. An early popular article on Ehrlich's syphilis cure, Salvarsan. (The Outlook, March, 1911.) This is a chemical cure, an arsenic compound, which (like quinine in malaria) has a greater affinity for the parasite than for the human tissues.

3. A reliable description of the arsenic cure for syphilis, (Science, Dec. 9, 1910).

4. Various tests for syphilis. What is meant by an enzyme test? Does it differ materially from the differential tests mentioned earlier in this course, e.g., the liquefaction of gelatine, or the fermentation of a selected sugar (saccharose)?

5. Organism causing gonorrhoea; see illustration, also.

6. City and state regulations aimed at the prevention of blindness (from gonorrhoea).

7. The Social Disease and How to Fight It, Creighton, (Longmans). This and the following are probably the sanest of the books dealing with the practical phases of this question.

8. A New Conscience and an Ancient Evil, Addams, (Macmillan).

9. Sex-Education, Bigelow, (Macmillan, 1916). This discusses all phases of the sex-education movement; invaluable for teachers.

10. A list of the references on sex education (including sex hygiene) may be secured from the instructor; much valuable material may also be obtained through the Society for Moral and Social Prophylaxis, New York City (e.g., The Relation of Social Diseases to the Family, by Prince Morrow).

11. Typical pamphlets for the information of the public:
    - a. Venereal Disease Number, (Health News, May, 1917).
    - b. Common Causes of Blindness in Children, (No. 1, Nat'l. Com. Prevention of Blindness, N. Y. City).
    - c. The Relation of Social Diseases to the Family, Prince A. Morrow, (Am. Journ. of Sociology, Vol. 14, p. 622, 1909).
    - d. Social Hygiene and the War, W. F. Snow, (No. 108, Am. Social Hygiene Assn., 1917).
    - e. Social Hygiene; the issue for October, 1917, illustrates the work of the society, the Am. Social Hygiene Association.

XVII. ANIMAL PARASITES

(MAINLY PROTOZOA)

(This topic is covered mainly by the following demonstration.)

1. The emphasis so far has been upon bacteria rather than protozoa. This is due to three reasons:
    - a. Bacteria are more important in economic processes and relationships than protozoa.
    - b. In this latitude more of the human diseases are due to bacteria than to protozoa.
    - c. Protozoa are more difficult to cultivate on ordinary laboratory media than bacteria are.

2. In general, the statements made concerning bacteria (their vital phenomena, the effects of physical and chemical agents, the reactions of the body, etc.) hold for protozoa also.

Morphologically protozoa differ from bacteria in several respects:
- a. they are usually much larger;
- b. the body is more flexuous;
- c. the nucleus is easily distinguished;

d. the life history is often more complex: many protozoa pass through a definite life cycle in a single host or substrate, or in alternate hosts.

3. Other small animal organisms higher than the protozoa are often the cause of disease. Many of these belong to the round worms.

Look through the marked pages in Stitt (Practical Bacteriology, Blood Work, and Parasitology, Blakiston) for some of these types.

4. a. This microscope shows trichina, coiled or encysted worms, lying between the slender striped muscle cells.
   b. See marked passages — Trichinosis, Ransom, (Circ. 108, Bureau An. Ind., 1907).

5. a. Hookworm is also a round worm. The second one described here, *Necator,* is the American one, found in our southern states. The remedy is a simple one, thymol followed by Epsom salts.
   b. To get an idea of the hookworm problem, look through these marked pamphlets:
      (a) Hookworm Disease and the Negroes, Stiles, (No. 9, Hampton Leaflets, 1909).
      (b) The Rural School and Hookworm Disease, Ferrell, (Bull. 20, Bureau of Education, 1914).

6. A simple classification of protozoa. The groups containing the species of special interest to us are marked. Most protozoa are not pathogenic to man and the important domestic animals.

7. a. Look through these books containing pictures and descriptions of some of the protozoa: Calkins, The Protozoa, (Macmillan); Stokes, Infusoria, (Trenton Natural History Society).
   b. These books (Park, Jordan, Moore, and Stitt) picture many of the protozoa pathogenic to man and such animals as the cow, horse, and sheep. Find three of the illustrations and legends which indicate a definite life cycle; also three protozoa which inhabit alternate hosts.

8. These amoebae illustrate three striking differences between bacteria and most of the one-celled animals; note the larger size, the distinct nucleus, and the more flexuous body, with its changing "pseudopods." Locate the *solid* particles of food that these amoebae have ingested.

9. Much interest lately has centered around the mouth amoebae and their rôle in dental decay. See the accompanying article, Significance and Prevention of Amebic Infections in the Mouths of Children, Williams and others, (Reprint 37, Department of Health of the City of New York).

10. Paramoecium (×60), another of the protozoa. Note the large size and the distinct nucleus. Some of these are conjugating; bacteria have no sexual phase like this.

11. Trypanosomes in dourine, a blood disease of horses.

Note how numerous they are — more numerous than the red corpuscles themselves. Compare with the illustration. This organism, *Trypanosoma equiperdum,* causes heavy losses to horse breeders in Europe and the northwestern part of our country and Canada. (Paralysis characterizes its development.)

12. Trypanosomes from the blood of a rat. Note how distinct the nucleus is.

13. Spirochaetes are more slender than trypanosomes, and the body consists of more curves or spiral turns.

The spirochaete shown (relapsing fever) was the first one definitely related to disease.

14. *Coccidium,* a little protozoan that causes various diseases in animals. It often affects markedly the liver and lungs, and causes in them a dotted appearance that can be seen with the naked eye. This slide of bile (rabbit) has a general red background; the coccidia are the large blackish, somewhat elliptical bodies (×80).

15. Mashed bit of brain tissue showing 3 kinds of bodies: (1) the round to oval, dark blue ones; (2) slightly *larger* reddish blue ones and (3) still larger, brighter red bodies, more porous-looking. See labelled illustration, and identify

blood corpuscles, nuclei of disintegrated nerve cells, and Negri bodies, (cause of rabies).

16. *Eimeria,* small rounded protozoa (×60) in the tissue of a chicken. This illustration shows the interesting life cycle changes.

17. Texas fever is caused by a small one-celled animal, *Piroplasma.* See the illustrations. This organism has two alternate hosts, (1) the cow and (2) the tick, which attaches itself to the body of the cow.

18. a. In malaria the alternate hosts, mosquito and man, are less intimately associated than the hosts of the Texas fever parasite. The life cycle is apparently much more complex. See the accompanying diagram. Which stage is shown in the blood slide under the next microscope?
    b. A popular account of mosquitoes and their connection with yellow fever, malaria, etc. The organism causing yellow fever has never been seen. What reason have we for thinking it is a protozoan?
    c. Some Facts about Malaria, Howard, (Farm. Bull. 450, 1911).

19. Mallory and others claim that scarlet fever is due to a protozoan which may be found in the white corpuscles in certain stages of the disease; the accompanying illustration shows white corpuscles containing such "inclusion bodies."

20. The Protozoan Parasites of Domesticated Animals, Crawley, (Circ. 194, Bureau An. Ind., 1912).

XVIII. CHRONOLOGICAL LIST OF MICROBIOLOGICAL INTEREST

(This is not meant as a complete history; these abbreviated statements include usually only the *first* aggulutinin, the *first* spirochaete, etc.)

Early theories and practices related to disease
— evil spirits
— humoral theory
— early rites and rules concerning foods, isolation, purification
1276 — First ruling — city slaughter houses
1510 — Sanskrit mosquito theory
1546 — Fracastorius — theories concerning disease
— true nature of contagious organisms
— disease transmitted — immediate contact
— intermediate agents
— air
— organisms — "glutinous" nature
— capable of reproduction
— made pathogenic through heat of animal body
1590 — Jansen — first microscope
1659 — Kircher — first to see and describe bacteria
— formulated theories concerning — infection
— transfer by flies
— wide distribution of infectious organisms ("worms")
1675 — Leeuwenhoek — first pictured bacteria (1683)
1688 — Redi — first statement of biogenesis; compare early statements (Aristotle, etc.) and later arguments concerning spontaneous generation

1745 — Needham — "proved" spontaneous generation
1769 — Spallanazani — air required for spontaneous generation
1836 — Schultz — no spontaneous generation with chemically-filtered air
1839 — Schwann — none with heated air
1854 — Schroeder & von Dusch — none with cotton-filtered air
1860 — Hoffman — none in flasks opening downward
1861 — Pasteur — Chevreuil and Pasteur flasks
— boiling does not kill all organisms
— putrefaction and fermentation not causes of spontaneous generation, but themselves due to metabolic activities of micro-organisms
1876 — Tyndall — origin of organisms in decomposing substances
1718 — Inoculation against smallpox
1745 — Needham — "proved" spontaneous generation
1762 — Plenciz — theories — all infectious diseases due to micro-organisms
— period of incubation explained by *living* organisms
— specified germs for each disease
1769 — Spallanazani — air necessary for spontaneous generation
1782 — Canning of vinegar introduced by a Swede
1786 — First classification of bacteria — vibrios: bacillus, lineola, and spirillum
1786 — Müller — morphological details regarding bacteria
1787 — Wollstein — glanders by inoculating pus
1795 — Gordon — puerperal fever contagious
1796-8 — Jenner — vaccination against smallpox
1804 — French process for canning — corked jars set in boiling H_2O
1807 — Gram rust a mold disease
1810 — Durand — English patent for canning in glass and tin
1819 — Daggett & Kensett — canning fish, oysters, etc.
1820 — Underwood & Mitchell — fruits canned — glass, tin
1834 — Causes of thrush (mold) and itch (mite) determined
1836 — Schultz — chemically-filtered air prevents spontaneous generation
1837 — Bassi — protozoan silk worm disease discovered
1838 — Ehrenberg — achromatic objective
— detailed study of bacteria, protozoa, and intermediate forms
1839 — Schwann — heated air prevents spontaneous generation
— yeast studied; cause of fermentation
1839 — Favus — a mold disease
1840-7 — Henle — relation of microscopic organisms to infectious diseases formulated
1841 — Fuchs (and Ehrenberg) — blue and yellow milk bacteria investigated
1841 — Valentine — first blood parasite seen (in fish)
1843 — Holmes — puerperal fever transmitted by medical attendants
1844 — Immersion lens
1847 — Semmelweis — control of puerperal fever — disinfectants
1848 — Nott — yellow fever theory
1849 — Pollender — anthrax found in blood of diseased animals
1850 — Davaine — anthrax in blood and in infections
1854 — Broad Street well epidemic of cholera worked out
1854 — Schroeder & von Dusch — no spontaneous generation with cotton-filtered air
1857 — Nageli — bacteria classed as plants — (Leidy? 1849)

1860 — Hoffman — flasks opening downward — no spontaneous generation
1861 — Pasteur — some organisms survive boiling
— increased temperatures (110-112° C.) effective
1863 — Davaine — inoculated anthrax blood; first bacteria definitely shown to be cause of disease
1865 — Pasteur — silk-worm investigation
— spores — reproductive bodies
— specificity of organisms
1865 — Tuberculosis induced by Villemin — by inoculating affected tissue
1866-71 — Micro-organisms demonstrated in wound infections (Klebs, Rindfleisch)
1870-72 — Bacteria seen in white corpuscles
1870 — Abbe condenser
1870 — (1863-70) — Lister — antiseptics, and aseptic methods; effect upon surgery
— high dilution method of obtaining pure cultures
1871 — Bacteria stained — Weigert and others (carmine, etc.)
1871 — Leprosy seen
1871 — Bacteria present in lymph in erysipelas
1872 — Pasteur — specificity of bacteria — pigmented forms
1872 — Cohn — systematic classification: micrococcus, bacterium, bacillus, vibrio, spirillum, spirochaete
1873 — Obermeier — first pathogenic spirochaete — relapsing fever
1874 — Panum — vague theory of white corpuscle action
1874 — Traube and Gescheidal — fresh blood bactericidal
1874 — Hall — milk obtained sterile
1876 — Klebs — special media — hen's eggs
1876 — Tyndall — origin of bacteria in decomposing substances
1876 — Cohn — spores but a phase in the life of certain organisms
— resistant to heat
1877 — Aniline dyes — Weigert and Ehrlich
— special stains — Koch and Gram
1877 — First protective serum — Raynaud — on calf
1877 — Woronin — protozoan disease of cabbage
1878 — ? Klebs — inoculated monkeys with syphilitic material; Metchnikoff, 1878
1878 — Term "microbe" first used
1879 — Thermophile bacteria, growing at 65-76° C.
1879 — Gonococcus seen
1880 — Zeiss objective perfected
1880 — Pasteur — fowl cholera bacillus isolated
— immunity by attenuated virus (age): fowl cholera, anthrax
1880 — Laveran — malaria — protozoa in blood
1880 — Pneumonia seen by Sternberg and by Pasteur
1881 — Koch — solid media for obtaining pure cultures; purity uncertain before
1881 — Klebs — typhoid
1882 — Attenuation by chemicals — Roux & Chamberland
1882 — Koch — tuberculosis — pure culture
— 4 laws for proving a given organism cause of a specific disease
1882 — Pasteur — rabies; cure by attenuated form
1882 — Glanders — pure culture; inoculation experiments
1882 — Metchnikoff — action of white corpuscles
1883 — Bacterial diseases of plants; pear bacillus of Burrill

1883 — Diphtheria seen by Klebs
1883 — Cholera isolated by Koch
1883 — Bone decay due to bacteria
1883 — Erysipelas — a streptococcus infection (earlier (1882) in abscesses)
1884 — Diphtheria — pure culture by Löffler
1884 — Typhoid — pure culture
1884 — Tetanus — produced by inoculation with affected blood
1885 — Tetanus — from inoculation with soil
1885 — Gonococcus — pure culture
1887 — Meningitis — pure culture
1887 — Malta fever organism (coccus) discovered
1888 — Nuttall — bactericidal effect of blood serum
1888 — Winogradsky — nitrifying bacteria
1888 — Yeast — one cell method of securing pure cultures
1889 — Tetanus — pure culture — anaerobic methods
1889 — Texas fever — protozoan; alternate hosts
1890 — Behring — and Roux independently — antitoxin — diphtheria
1890 — Smith — sterile tissue as culture medium
1892 — Influenza bacillus — pure culture
1892 — Behring and Kitasato — tetanus antitoxin
1894 — Bubonic plague bacillus — Kitasato; Yersin
1895 — Diphtheria antitoxin first used by N. Y. City Board of Health.
1896 — Agglutinin — Gruber & Durham — cholera; *B. coli*
— Widal, as test for specific diseases
1897 — Precipitin by Kraus — plague and cholera
1898 — Dysentery bacillus isolated by Shiga; 1898-1903, various types by others
1898 — Pleuro-pneumonia cultivated — collodion sacs — peritoneal cavity — Roux & Nocard
1898 — Ross — malaria — relation to mosquito
1898 — Richet and Hericourt, anaphylaxis; Smith, 1904
1900 — Yellow fever; work of Reed, Carroll, Lazear, etc.
1902 — Opsonins — Wright, etc.
1903 — Protozoa cultivated — Novy, McNeil
1905 — Syphilis stained — Schaudin and Hoffman
1913 — Rabies organism identified; probably a protozoan
1913 — Infantile paralysis stained and cultivated; smallest known organism.
(See last paragraph, p. 5.)

XIX. APPENDIX

ADDITIONAL EXERCISES

Additional laboratory and demonstration exercises will be added if time permits in the various sections. Typical additions are given below. Under what main topic does each belong?

1. a. Anthrax grown in test tubes; note (1) that spores are present in this smear, and (2) that the organisms grow in long chains.

2. Anthrax from animal tissues (guinea pig liver, on a background of serum, etc.). These differ from those grown on artificial media in:

(1) lacking spores, (2) possessing *capsules,* (3) growing in short chains.

3. Gram stain. Try the Gram stain for one of your organisms. Use gentian violet (3 parts saturated alcoholic solution to 20 parts of 5% carbolic acid, as in carbol-fuchsin). Follow the accompanying directions (Moore's Microbiology). Organisms that keep this stain in spite of the decoloring power of alcohol are termed Gram positive; those that lose it, Gram negative. Which is your culture?

4. a. Organisms that lose their stain entirely may not be visible in the microscope. A weak stain (counter-stain) is often applied after decolorizing. This slide was counterstained with safranin, a red stain. Are the bacilli in it negative or positive? The cocci?

b. In the Gram stain, bacteria that resist the decolorizing power of alcohol, keeping their stain, may also be termed alcohol-fast. After staining this slide of saliva it was immersed in acid. (See text-book for directions.) Note that the tuberculosis organisms in this sample of saliva are acid-fast. This slide was "counter-stained" afterward with methylene blue. Are there any organisms in this sample of saliva which are *not* acid-fast?

5. a. Various articles on animal experimentation. Glance through the titles, noting the name and standing of the writer, too. Most of these are printed by the American Academy of Medicine, and may be obtained through that institution.

b. Compare these articles by "anti-vivisectionists" with the preceding set in (1) the standing of the authors; (2) evidences of direct knowledge and experience with regard to physiology, anatomy, surgery, etc.; and (3) scientific attitude and lack of prejudice.

REVIEW QUESTIONS

(The following list of review and examination questions may help the student realize just how much detail is expected in this course.)

1. Describe the complete life history of the bread mold (Rhizopus). Of a second mold.

2. Compare mold and yeast cells in structure and function with the ordinary green cells of the higher plants, (e.g., leaf palisade cells).

3. What is "compressed yeast"? Compare it with some other commercial yeast in methods of preservation, use, and structure.

4. Describe the general morphology of bacteria. To what is motility due?

5. Describe the process of making a "smear," explaining why each step is necessary.

6. How does hanging drop differ from an ordinary microscopic mount? What are the advantages secured?

7. Describe all the steps in transferring bacteria from one tube to another; making a plate count of water; in isolating a pure culture (throat). What do you consider the weakest place in the technique you have used in this class? Can you suggest desirable changes?

8. Describe briefly the various vital phenomena exhibited by bacteria. Which of these did you observe in studying yeasts?

9. How would you proceed to name any isolated bacterium?

10. List the useful bacteria — by name or preferably by use or results.

11. Which of these affect the food supply directly? indirectly?

12. What do you understand by the nitrogen cycle?

13. What is a check or control? Illustrate, and show why it is necessary in experimental work.

14. Define disinfectant, germicide, deodorizer, sterilization, and antiseptic.

15. Why does canning preserve fruit? why do "preserved" goods keep better than canned ones?

16. Define haemolysis, anaerobic, typhoid carrier, natural immunity, acquired immunity.

17. What are the uses of the streak plate, pour plate, blood pour plate, and "fishing"?

18. Name the important physical and chemical agents that affect bacterial growth. Are these inhibitory or germicidal? Illustrate.

19. What are the advantages of carbolic acid over other liquid disinfectants? Of formaldehyde, over other gases?

20. Compare the effects of dry and moist heat and gases on bacteria. What practical application do we make of this?

21. What pathogenic bacteria form spores? How does this affect the temperature necessary for sterilizing, disinfecting, pasteurizing, etc.?

22. Account for the different tastes of butter made by different farmers; by the same farmer at different times. How can uniform results be secured?

23. To what are the different kinds of cheeses due?

24. Why do we boil water, but only pasteurize milk? How may the disadvantages attending these processes be overcome? Wholly?

25. What is the theory or basis upon which water examination is conducted? What diseases have been definitely traced to water? What are the methods of safeguarding the drinking water supply?

26. Show that the milk supply of a city is within the province of the health department. Name the sources of contamination or infection to which the milk is exposed. What diseases are carried by milk?

27. Why are we justified in condemning milk upon a numerical count? Describe two methods of examining milk bacteriologically.

28. Discuss the mouth as a "third hand"; moistening the fingers with saliva before turning a page; ice *in* the drinking water; removal of rings *before* washing the hands; eggs with soiled or dirty shells; the use of rubber sponges; closed tooth brush holders; vacuum cleaners and methods of sweeping; why dried fruits keep; wiping or draining dishes; the habit of swallowing sputum; placing gloves, etc., on the dining table; why flower stems do not become slimy when kept in water in an icebox; good and poor methods of dusting; matting as a floor covering; the things a filter can *not* do; flies on the food; common drinking cups. Suggest five additions to this list.

29. Define toxin, antibody, antitoxin, agglutinin, opsonin, and bactericide, tuberculin, vaccine, and bacterin.

30. What substances are formed in the body to overcome disease or to counteract foreign substances?

31. Describe the work of the white corpuscles.

32. Name ten diseases caused by known bacteria; three by protozoa; two by molds; and four by organisms not yet isolated.

33. What are the four steps in the identification of an organism as the specific cause of a disease?

34. Show that it is as necessary to know how bacteria leave the body as how they gain entry to it.

35. What facts concerning tuberculosis are most vital to the general public? To the families or companions of such patients?

36. Describe fully the relation of mosquitoes to malaria.

37. The organism causing yellow fever has not been isolated; show that we are justified in holding mosquitoes responsible for the spread of that disease.

38. What facts concerning the sexual diseases (gonorrhea and syphilis) should every individual know? Every teacher?

39. Give briefly the theory upon which the following are based: vaccination to prevent smallpox; Pasteur treatment to prevent rabies; giving antitoxin to overcome diphtheria.

40. Name five prominent figures in bacteriology, showing the relation of each to the progress of the science.

41. What do you consider the strongest arguments for animal experimentation? Against it?

SAMPLE EXAMINATIONS IN BACTERIOLOGY

I

1. (50 min.) Discuss the milk question from a bacteriological standpoint. Give first a topical outline showing the relation of the parts of the discussion.

2. (20 min.) a. Give the best set of rules you can frame to protect yourself from infection (wounds and diseases in general).
   b. Suggest additional rules for others, aimed to insure for you complete protection.
   c. What gaps are still left? or what is necessary to secure the execution of (b)?

3. (30 min.) Define the following; select first ten demanding paragraph definitions, and define the rest in single sentences:

mycelium, spore, cytoplasm, denitrifying, protozoan, anaerobic, lactic acid fermentation, enzyme, respiration, photogenic bacteria, intracellular enzymes, optimum temperature, ultra-violet disinfection, sterilize, virulent, antibody, nitrogen cycle, antitoxin, agglutinin, presumptive test, differential medium, sand filter, legume nodules, bactericidal, alcohol, beer, vinegar, disinfect, toxin, septic tank, vaccine, infection, immunity.

4. (Additional and optional.) Discuss the economic phases of bacteriology which deal with the controlled growth or action of micro-organisms.

II

1. Make a tabulated outline of microbiology as it affects man (in its broadest sense); topics — not facts — are desired as final divisions of the outline.

Underline once (1) those you could use in teaching in high schools; or (2) twice those you consider essential to a housekeeper.

2. Discuss (1) the one most important to the U. S. as an agricultural country; (2) the one you feel most able to discuss fully.

III

1. (30 min.) a. Describe briefly the various methods of examining milk.
   b. the methods of securing a high grade milk supply or
   c. the regulations concerning the milk sold in New York City.

2. (10 min.) How is water examined? Include one presumptive test.

3. (25 min.) (a) Describe the nitrogen cycle; or (b) list the economic phases of microbiology, dealing with the inhibition of the growth and other activities of micro-organisms.

4. (10 min.) Give five rules of conduct based upon bacteriology that you feel are important for you.

5. (25 min.) Define or give the most important fact relating to the following: disinfectant, deodorizer, agglutinin, pasteurize, chlorophyll, objective, condenser, organic, canning, bacteria, protozoa, toxin, antitoxin, vaccine, differential medium, pathogenic, cheese, pour plate, aerobic, denitrifying, nitrifying, Pasteur, Koch, solid media, and either Semmelweis, Weigert, or Metchnikoff.

IV

(Answer either part of 2)

1. List in a column with brief descriptions or definitions all the evidences of life evinced by bacteria.

2. a. Diagram the nitrogen cycle, indicating the parts performed by bacteria.
   b. Make a similar cycle for some other element, such as carbon, oxygen, or potassium.

3. Name the best liquid (or gas) disinfectant for general use. Justify briefly your selection. How do disinfectants act? Give two ways in which their efficiency may be increased.

4. How would you treat the following?
—a freshly cut finger,
—an abscessed wound,
—dishes used by a diphtheria suspect,
—intestinal discharges of a typhoid patient,
—ready-made clothing that is not washable,
—a room previously occupied by a tuberculous person,
—clothing with a scarlet fever history which you wish to give away.

5. List seven to ten facts or principles that you consider all important for a teacher (or housekeeper), and which would make for safety, if no others were included.

6. Define
microbiology
bacteria
protozoa
plasmolysis
cell
nucleus
toxin
vaccine
antitoxin
antiseptic

7. Name ten important historical dates or facts in microbiology; or give four important contributions to the science and show their later influence on its development.

REQUIRED READINGS

All books for reference are in the H. A. Library (first floor); additional copies may also be found in this laboratory.

"Optional" means that you will find the reference valuable but not absolutely necessary (for passing the tests, etc.).

"Introductory" indicates simple (often high school) texts on which no notes are expected. They are very general in character, and are for the purpose of assuring that we all start with the same *elementary* foundation or background. Usually they can be read *very* rapidly.

1. Green Plants
    - (a) Curtis: Nature and Development of Plants. Chapter I on the leaf. (Advised, but optional: chapters 2 and 3 on stems and roots.)
2. Molds
    - (a) Conn: Bacteria, Yeasts, and Molds in the Home. Read Molds (introductory).
    - (b) Bigelow: Applied Biology. 252-268. Read the experiments carefully.
    - (c) Buchanan: Household Bacteriology. 55-68; 424-427; read rapidly, 70-84, spending most time on the pictures and their legends.
    - (d) Jordan (optional): General Bacteriology. See index for pathogenic molds.
3. Yeasts
    - (a) Conn: Bread Yeasts (introductory)
    - (b) Bigelow: 268-271. Read the experiments carefully.
    - (c) Buchanan: Household Bacteriology. 42-48; (read rapidly) 48-54; 235-243.
    - (d) Jordan (optional): See index for enzymes and fermentation changes.
4. Bigelow: 276-297, 300-315 as an introduction to the one-celled plants and animals that are directly related to man, his food, health, etc.

Select *one* text from *each* of the following assignments. Consult the index unless pages or chapters are given.

5. Bacteria (structure and development): Jordan, Park, Buchanan, Kendall (Bacteriology, Lea and Febiger, 1916), or Hiss and Zinsser.

6. Vital phenomena exhibited by bacteria: Jordan or Park

7. Effects of physical and chemical agents on bacteria: Jordan, Park, Kendall, Buchanan, or Hiss and Zinsser

8. Air, soil, and water: Buchanan, Marshall, Jordan, or Park

9. Milk: See reference list under milk

10. Economic microbiology: either
    - (a) Marshall, 335-466 or
    - (b) Buchanan, p. 203-215, p. 235-243; Chap. 22 and balance of Chap. 25 (optional); add also topics treated in class from Chap. 24 and from Chap. 26 through Chap. 30

11. Immunity; the reactions of the body to disease. As assigned or consult index and chapter headings in Jordan, Buchanan, Kendall, Moore, Park or Morrey. (Fundamentals of Bacteriology, Lea & Febiger, 1917).

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